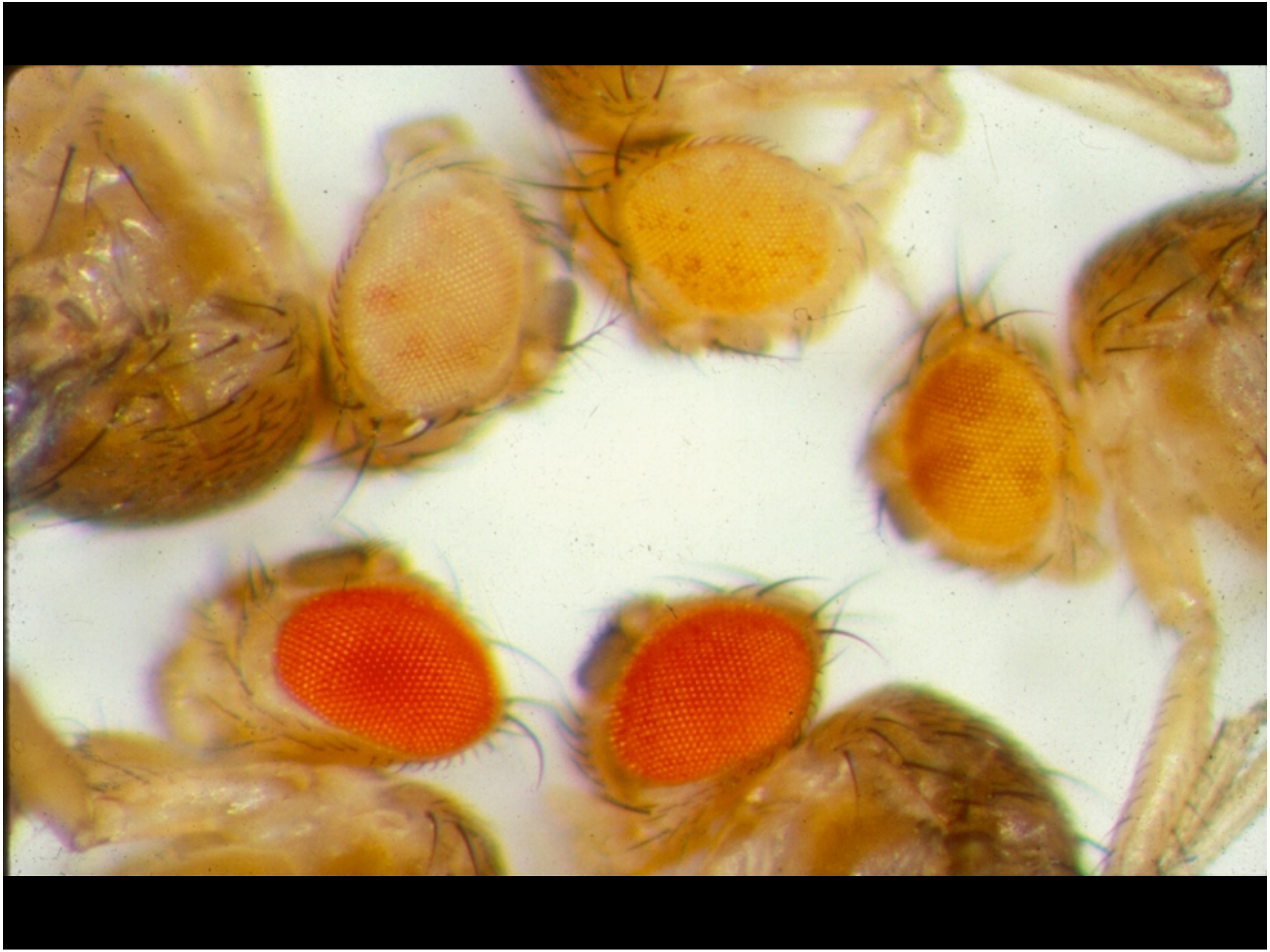
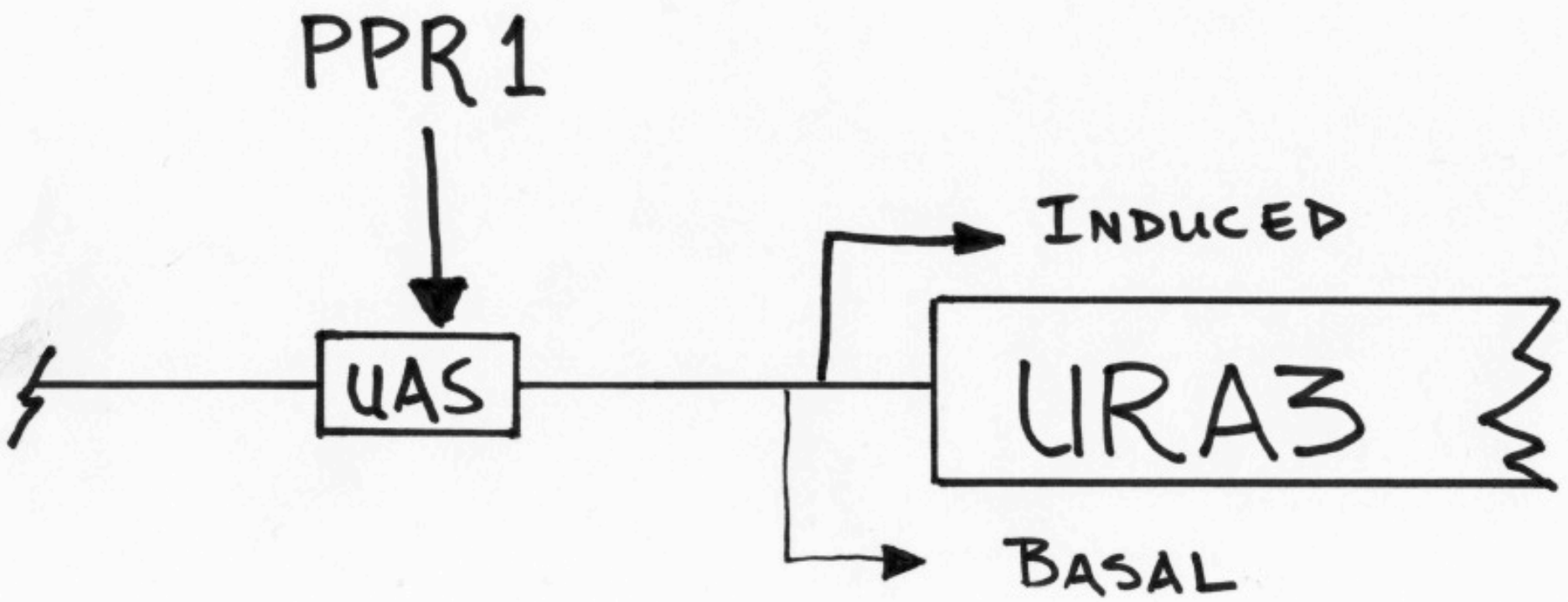


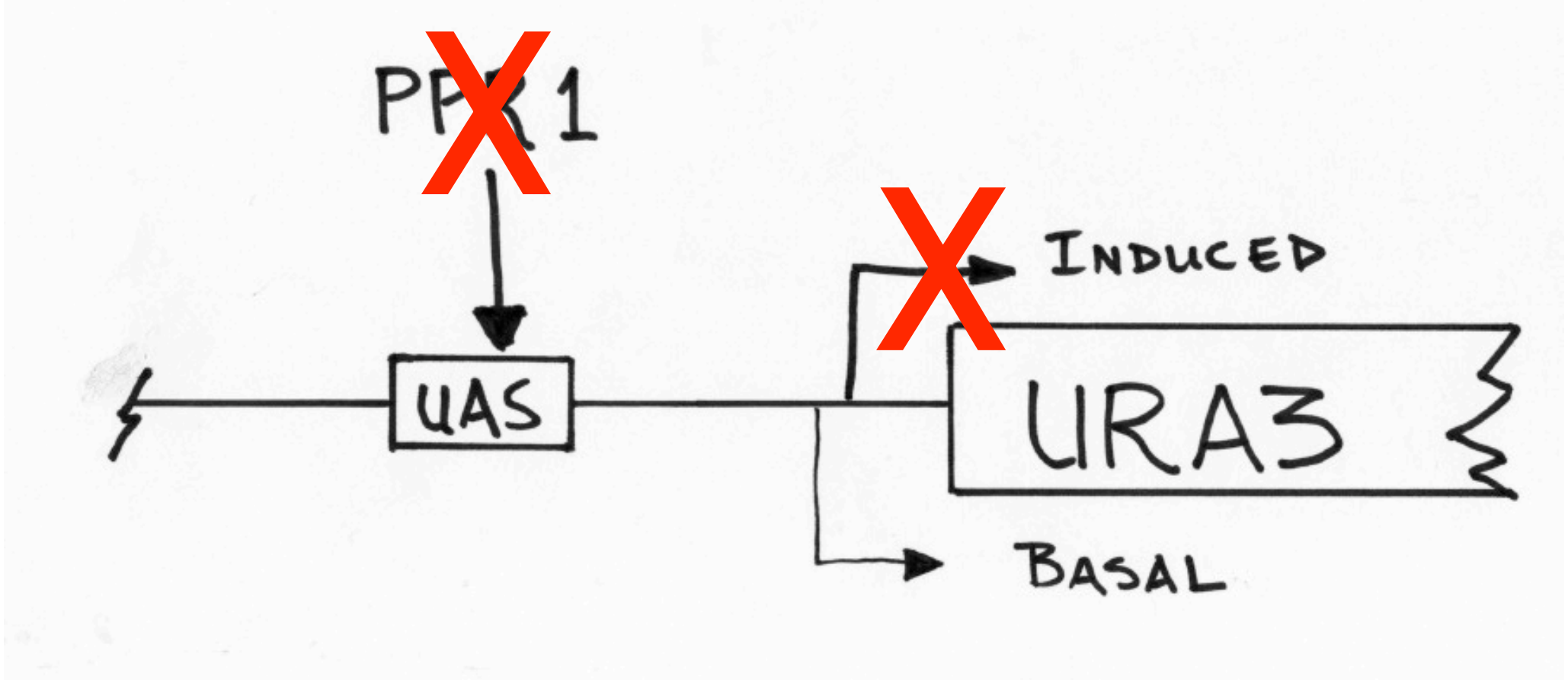
Positive & Negative Selection for URA3.

	-uracil	5-FOA
$URA3^+$	YES	NO
$ura3^-$	NO	YES

Positive & Negative Selection for URA3.

	-uracil	5-FOA
URA3^{+}	YES	NO
ura3^{-}	NO	YES


PPR 1
INDUCED
UAS
URA3
BASAL


PPR1
INDUCED
UAS
URA3
BASAL

Positive & Negative Selection for URA3.

Telomeric - *PPR1*

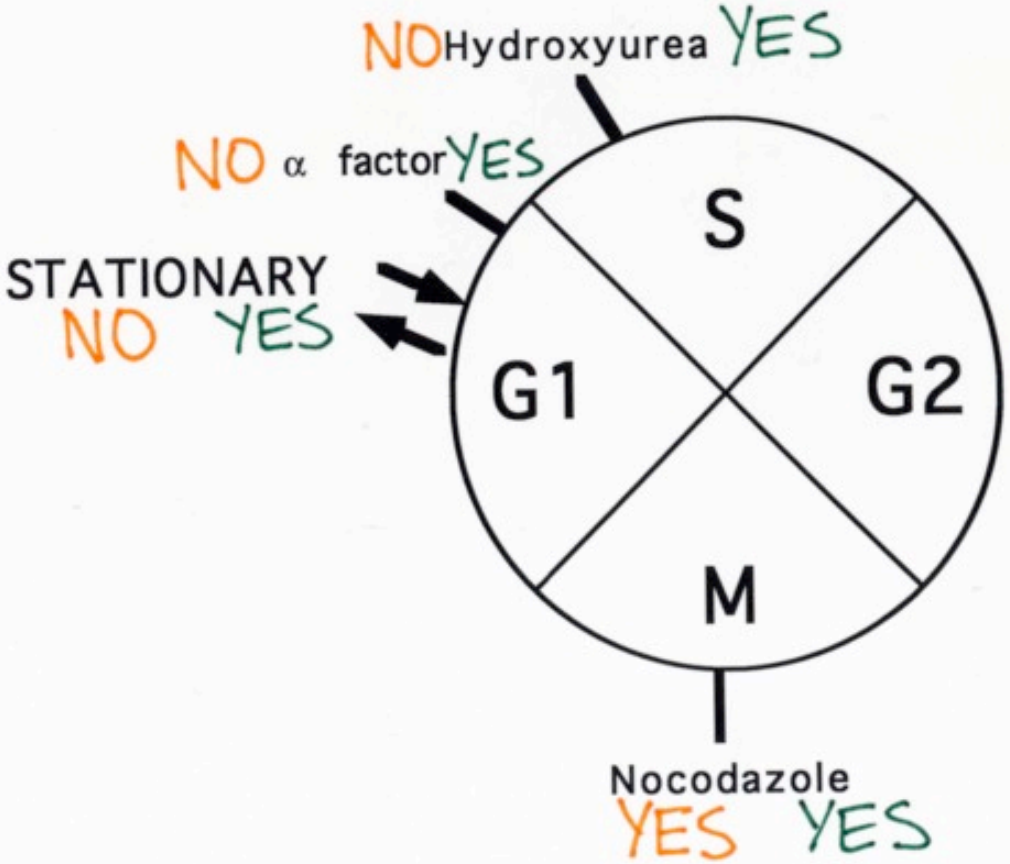
	-uracil	5-FOA
URA3^{+}	YES	NO
ura3^{-}	NO	YES

Positive & Negative Selection for URA3.

Telomeric - *ppr1*

	-uracil	5-FOA
URA3^{+}	YES	NO
ura3^{-}	NO	YES

WHEN CAN PPR1 ACTIVATE URA3 EXPRESSION?

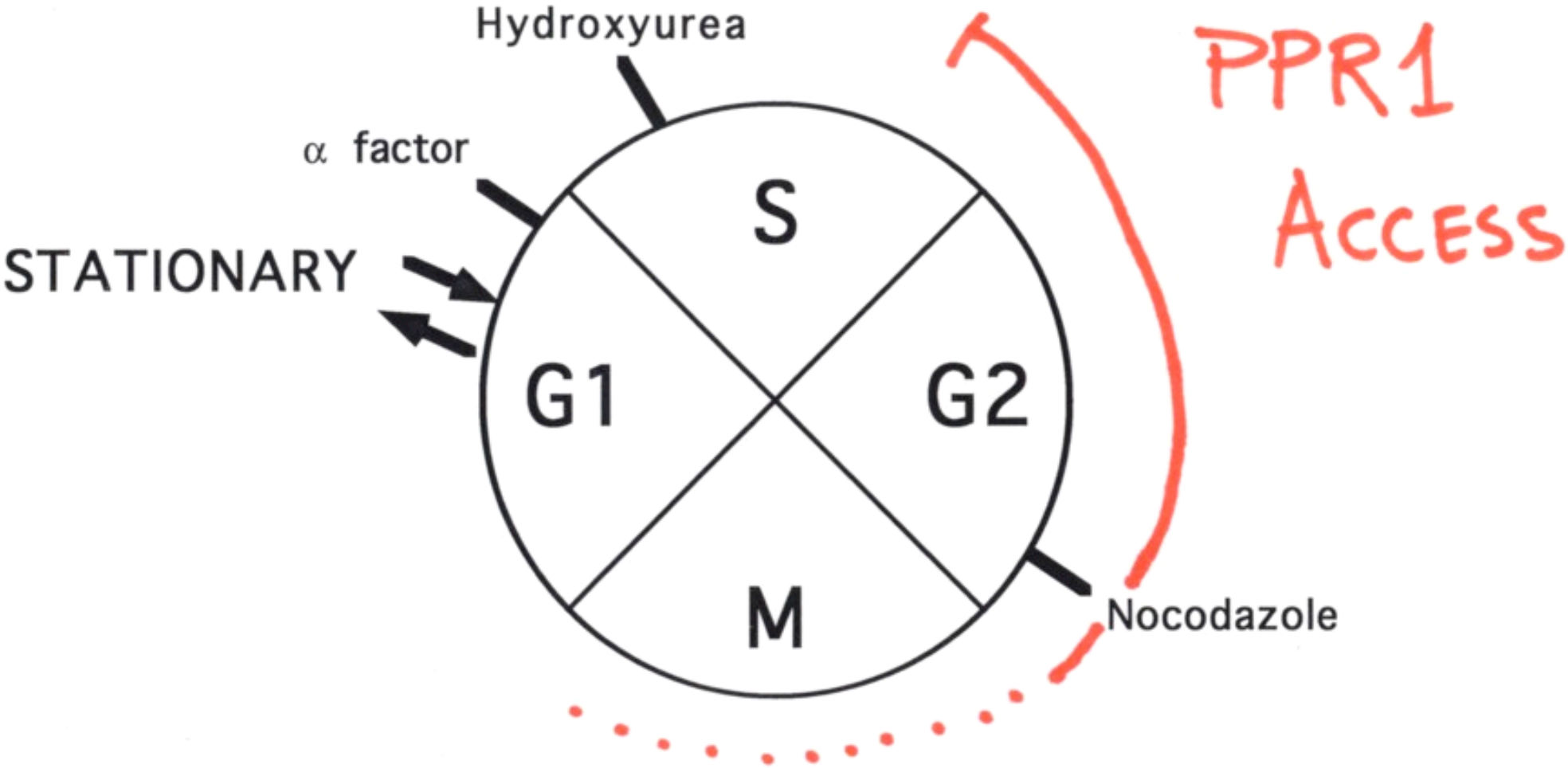


URA3 LOCATION

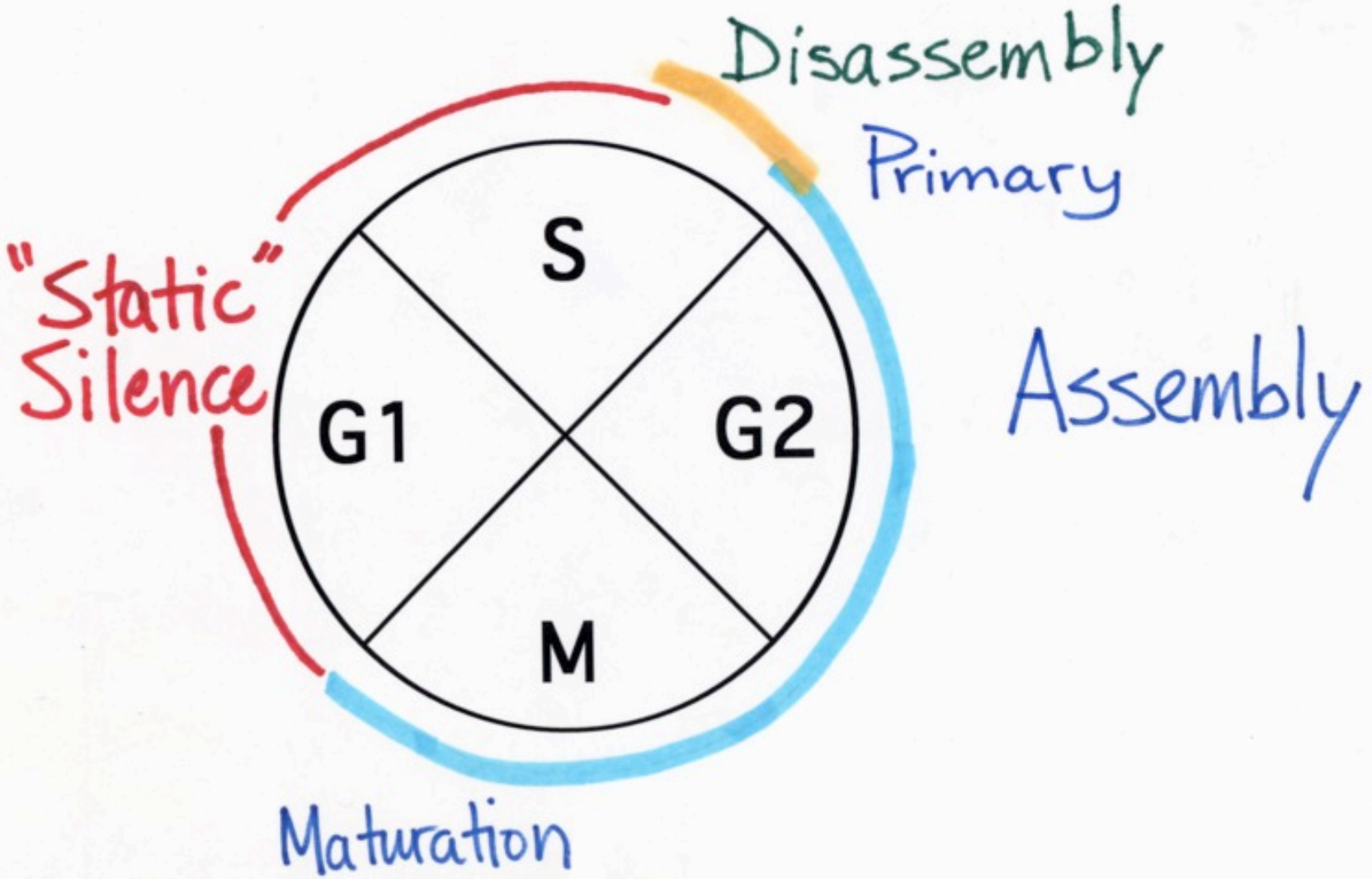
TELOMERE

INTERNAL

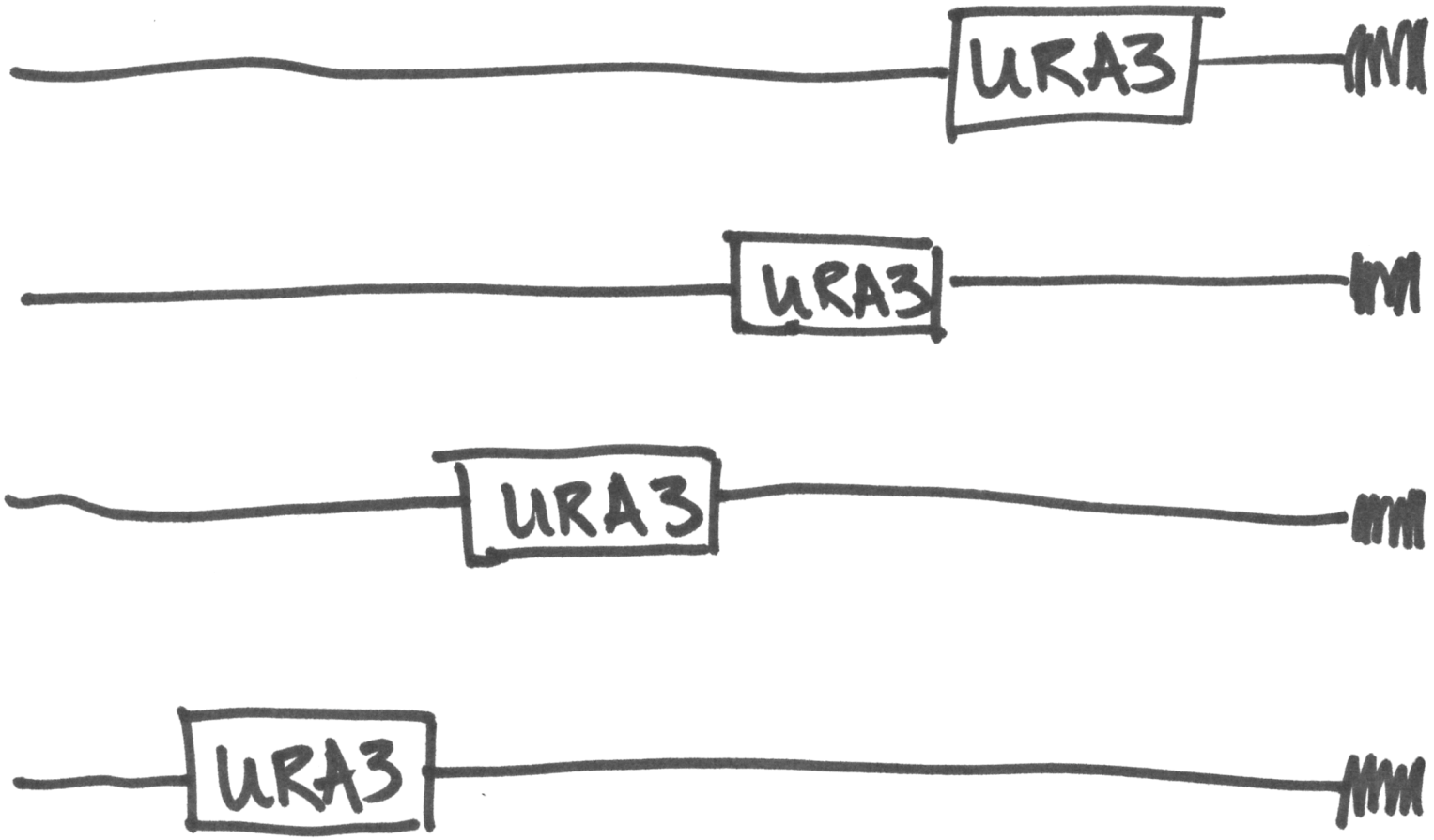
PPR1 has differential access to the telomere during the cell cycle



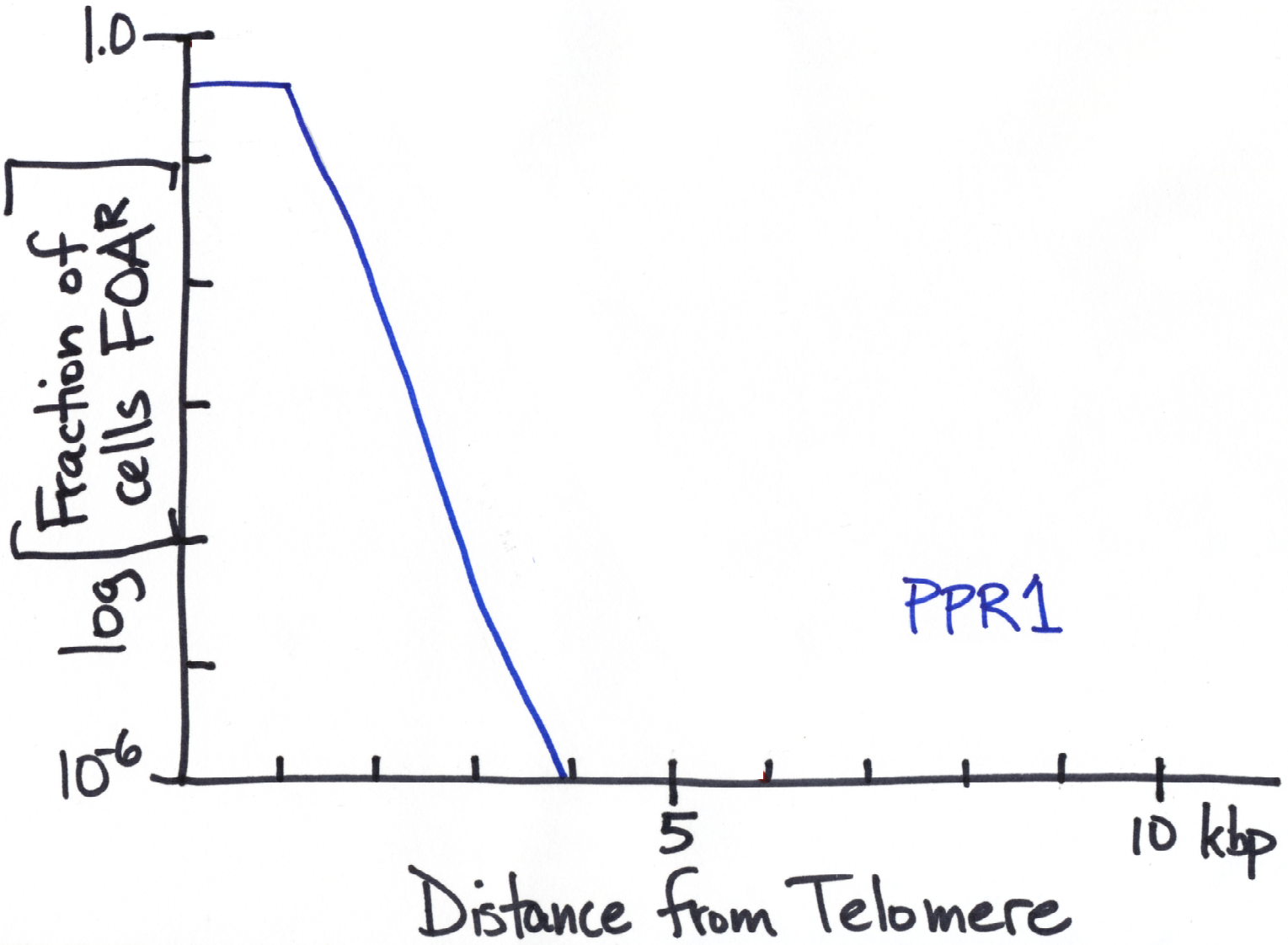
Dynamics of Telomeric Silencing



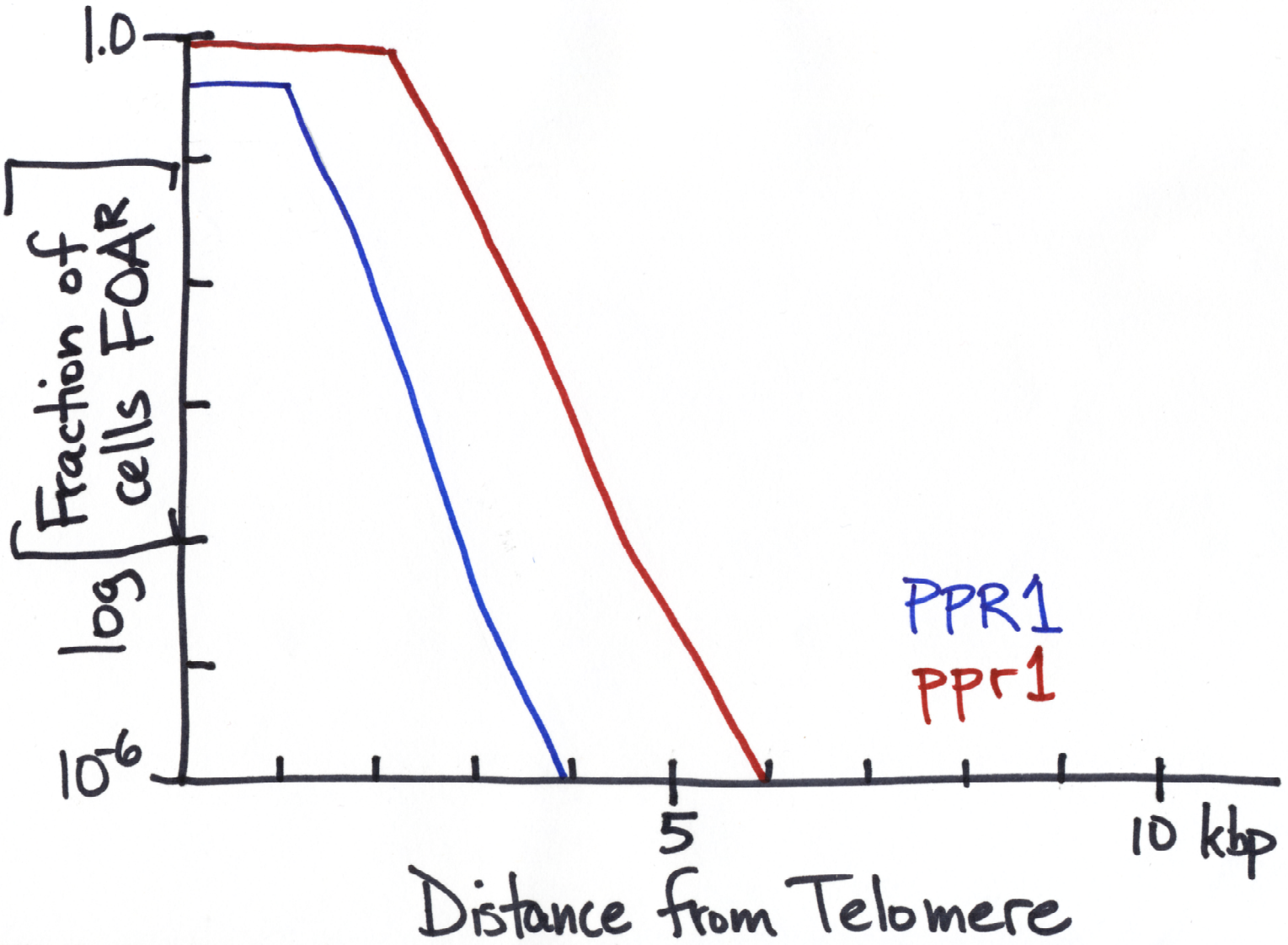
How far from the telomere does silencing occur?



Silencing "spreads" from the telomere

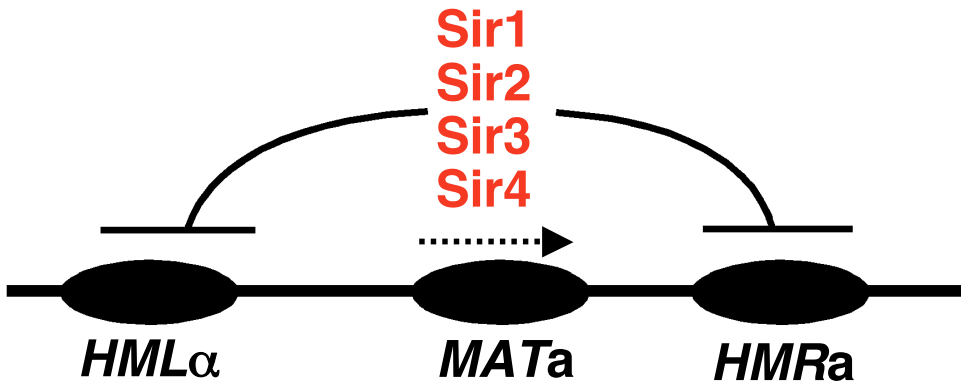


Silencing "spreads" from the telomere



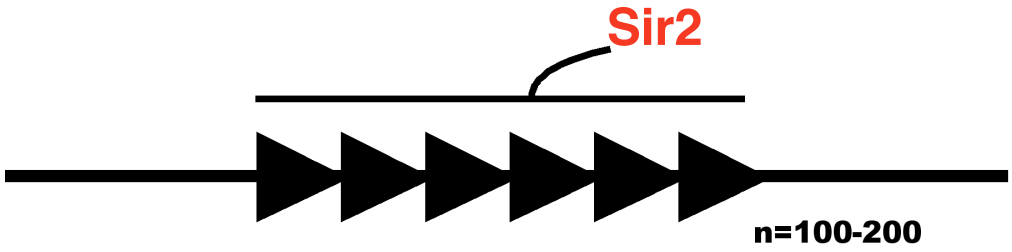
Gene Silencing in *S. cerevisiae*

Mating-type loci



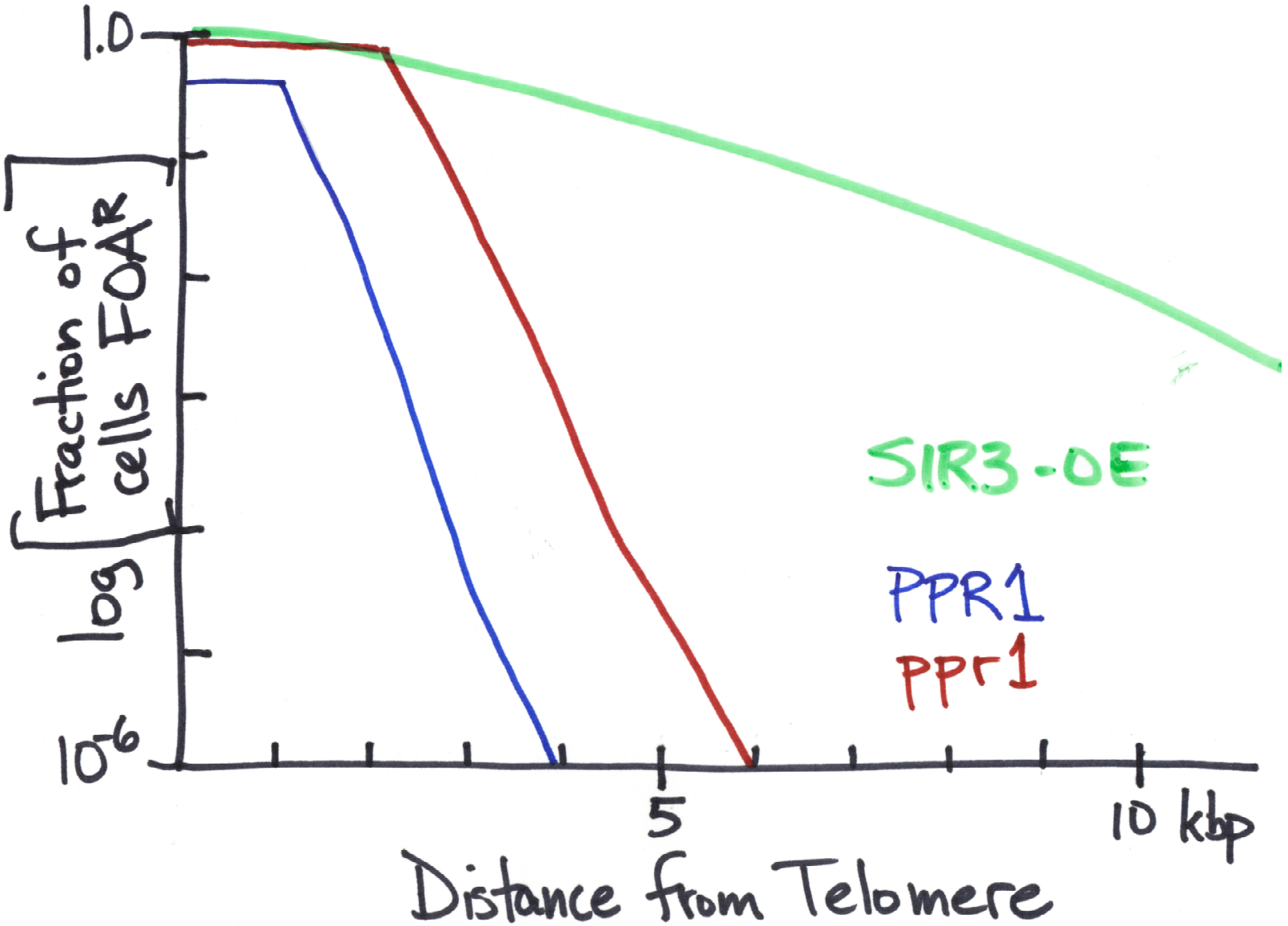
Telomeres

rDNA

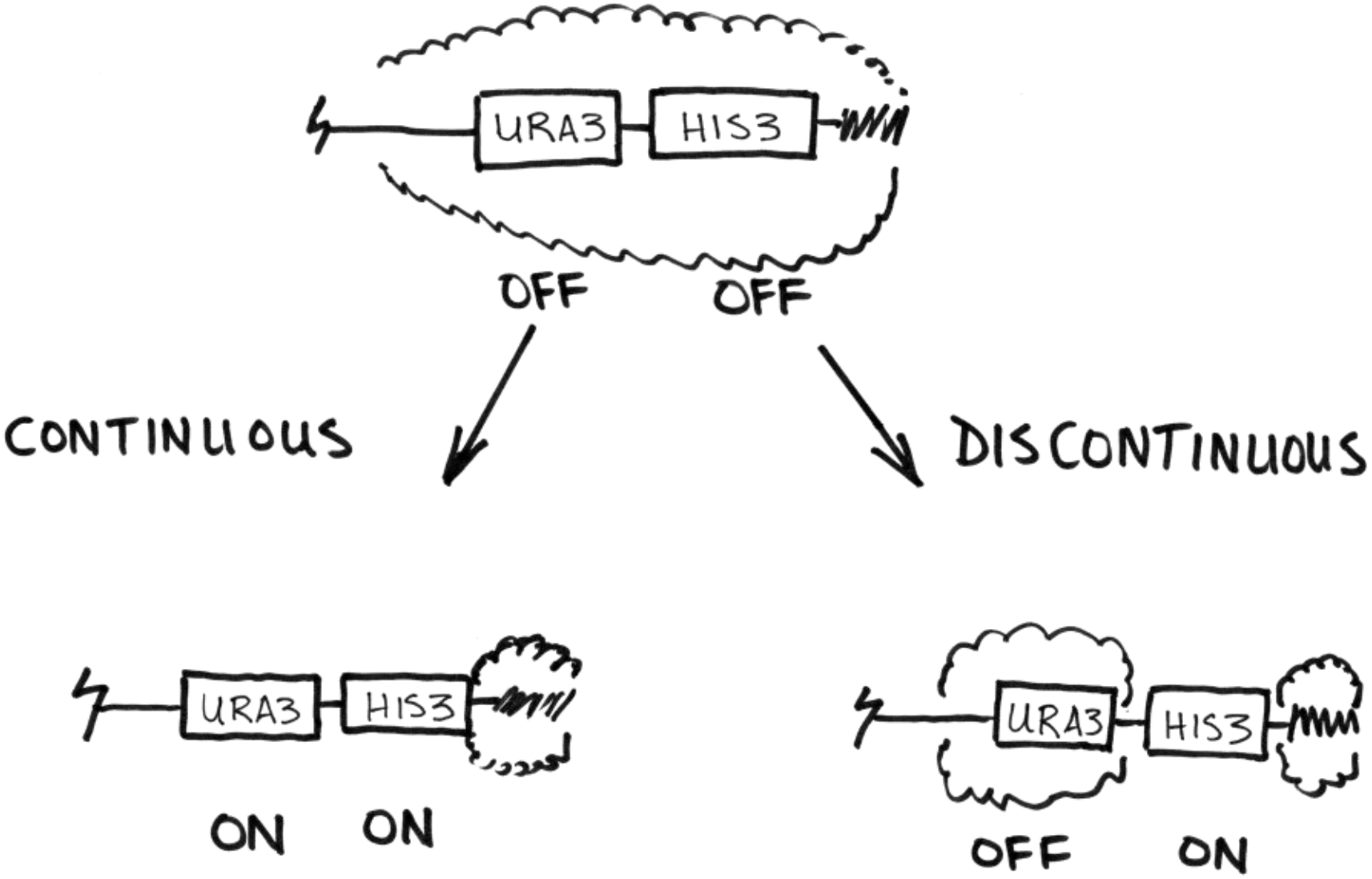


(~10% of the yeast genome is silenced)

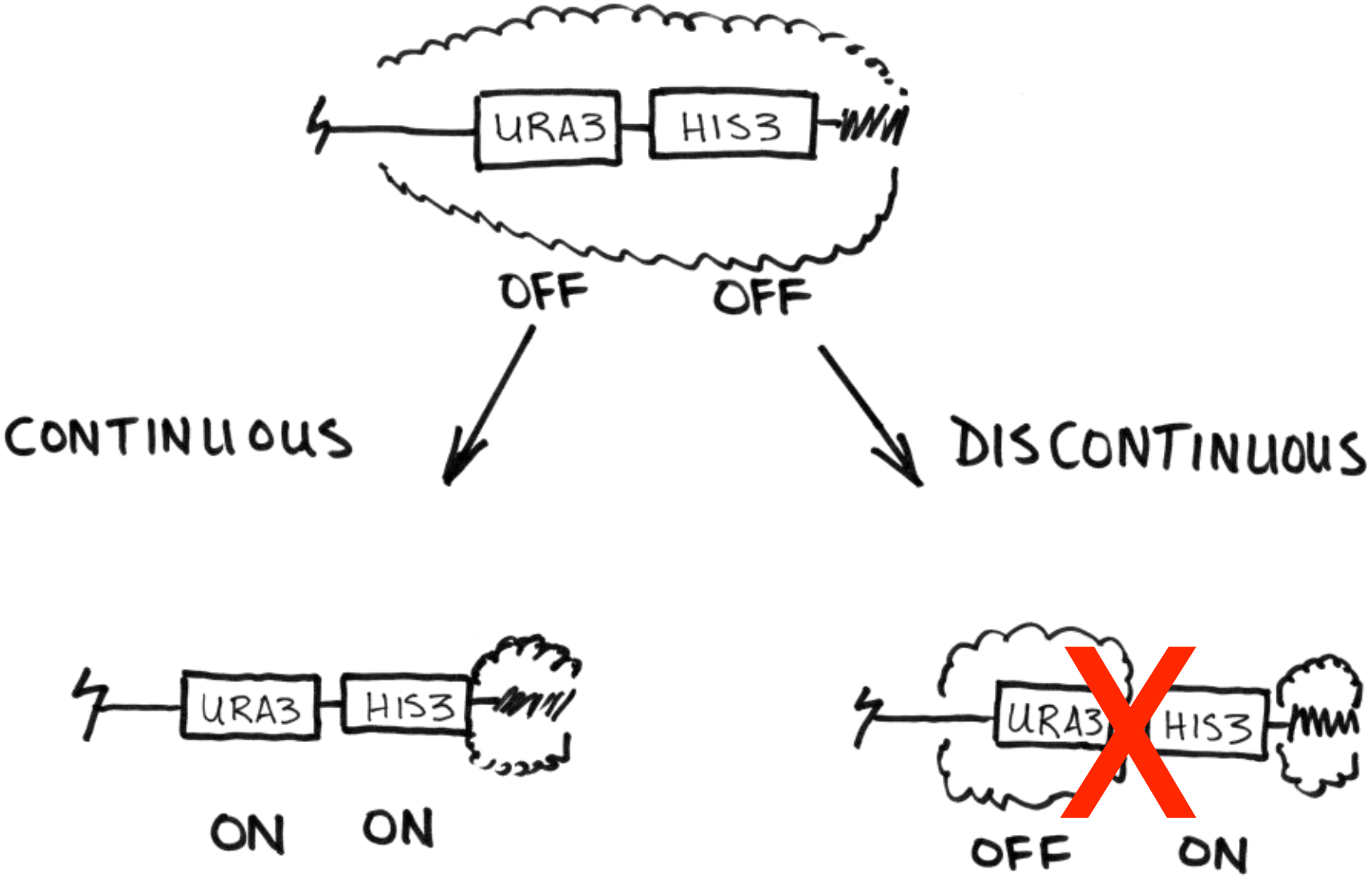
Sir3 is limiting for the spread of telomeric silencing



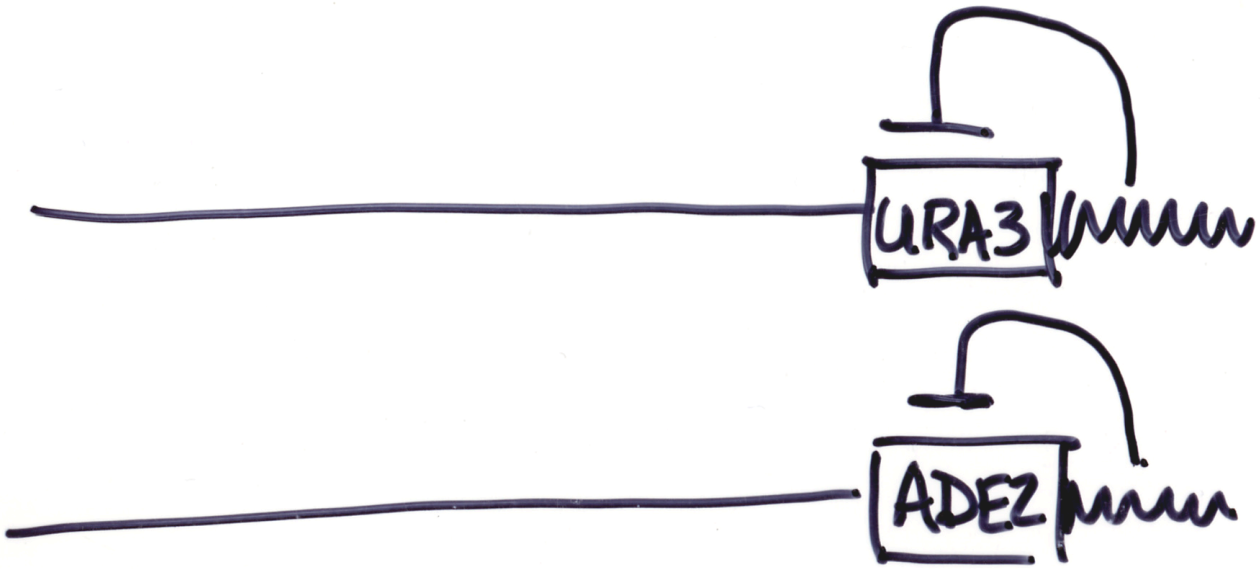
Does telomeric silencing spread continuously?



Does telomeric silencing spread continuously?



Telomeric Silencing



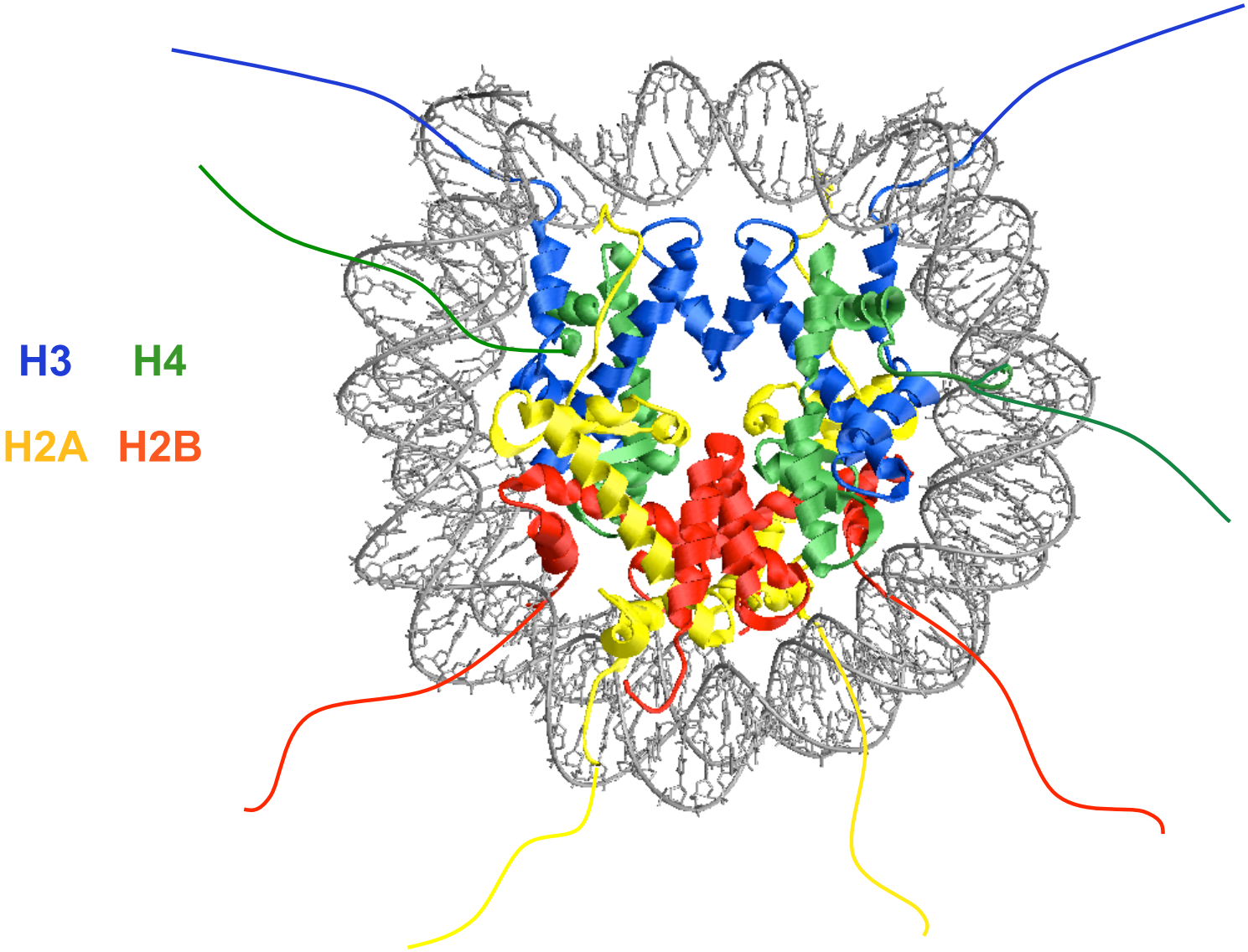
Ura⁻

Red/white sectored colonies

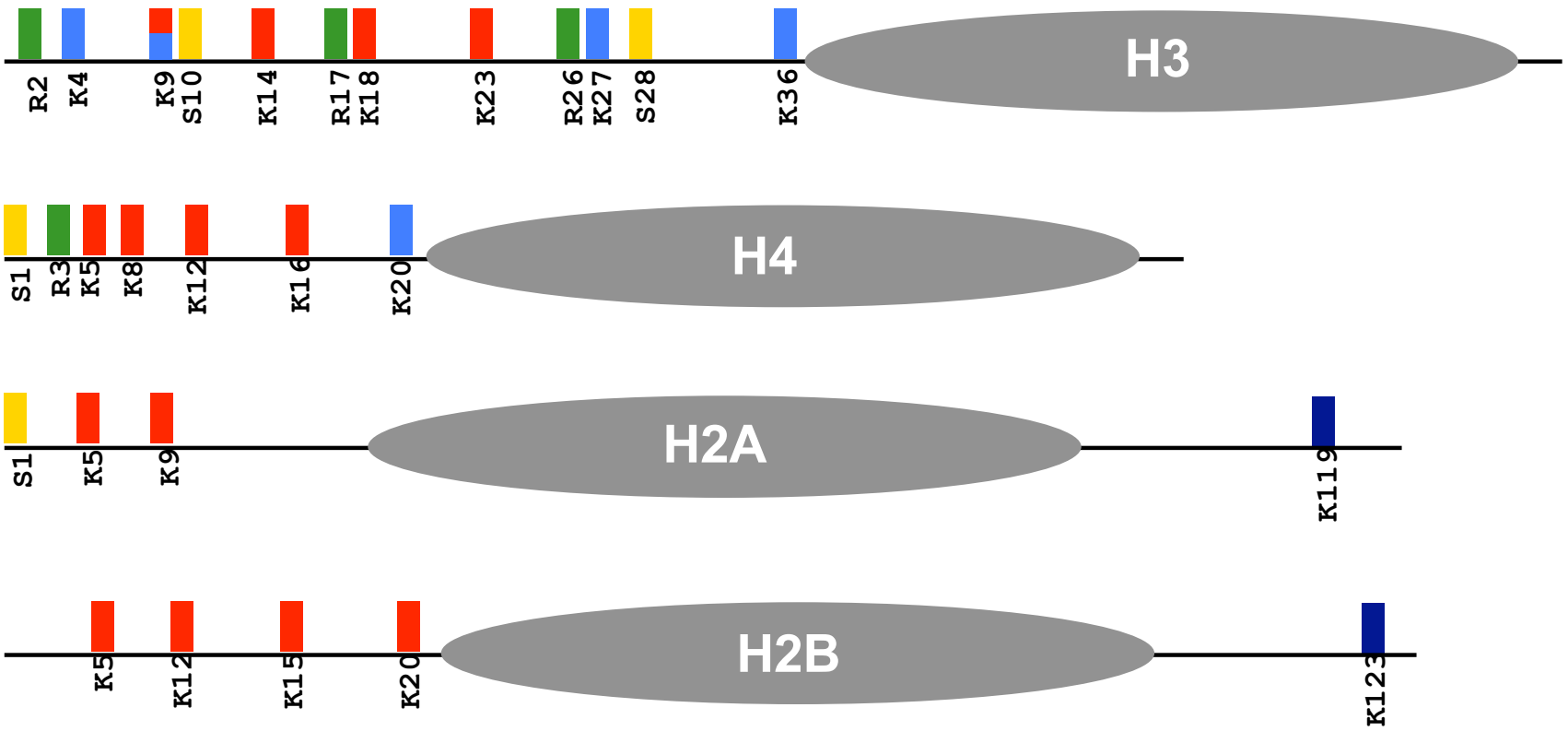
The multi-valent nature of telomeric silencing

- Rap1-> telomeric DNA repeats
- Yku70 & Yku80 -> DNA ends
- Sir2p - histone deacetylase
- Sir3p -> histones (unmodified)
- Sir4p -> Rap1, Ku & histones(?)
- Nucleosomes - H3 & H4 - via NH_2-tails & core - H2A & H2B-?

The Nucleosome: The Building Block of Chromatin

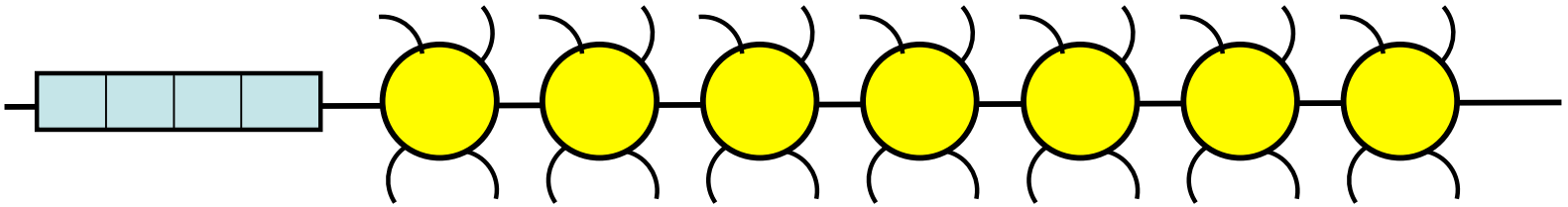


Histone tail modifications



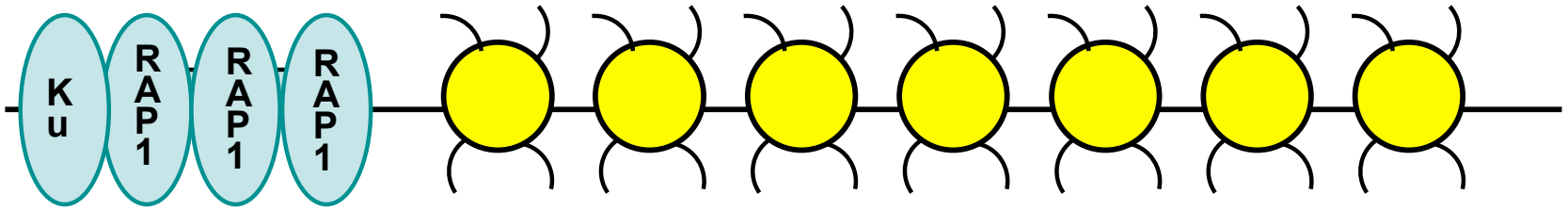
R methylation K methylation acetylation phosphorylation ubiquitination

Building Silent Chromatin

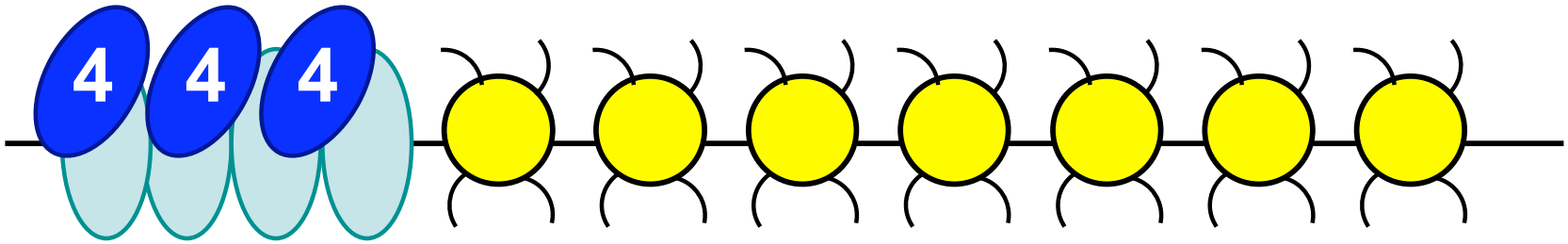


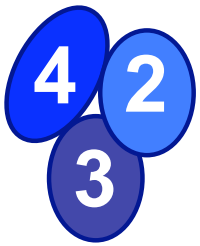
Silencer
(telomeric DNA)

Building Silent Chromatin



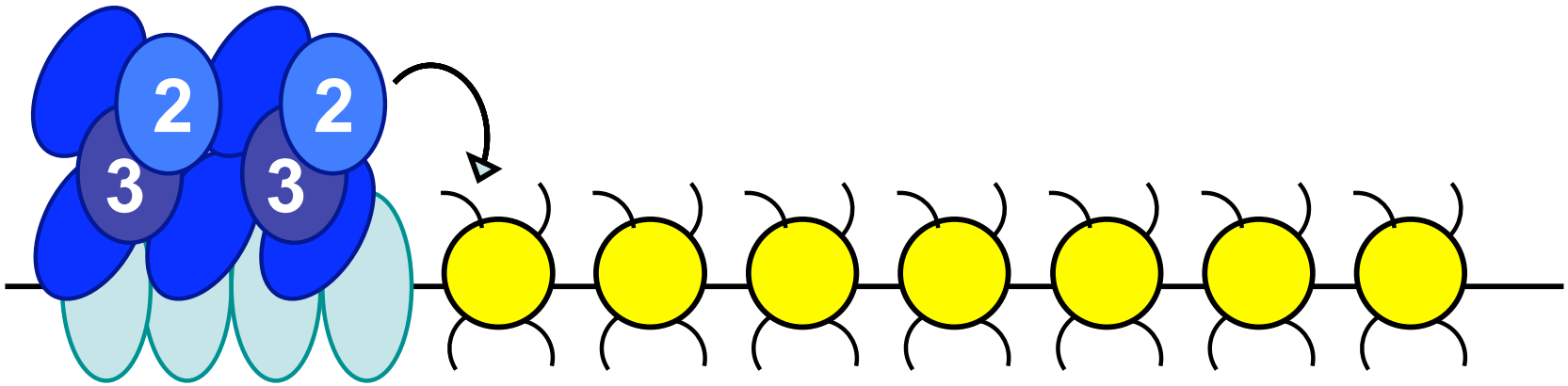
Building Silent Chromatin

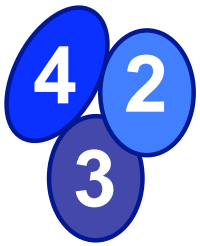




SIR complex

Building Silent Chromatin



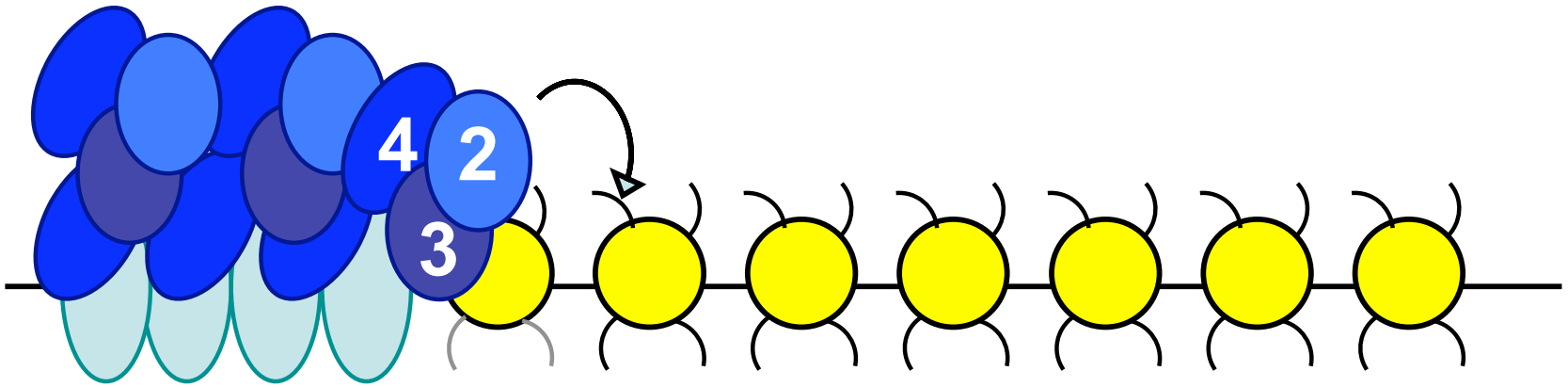


SIR complex

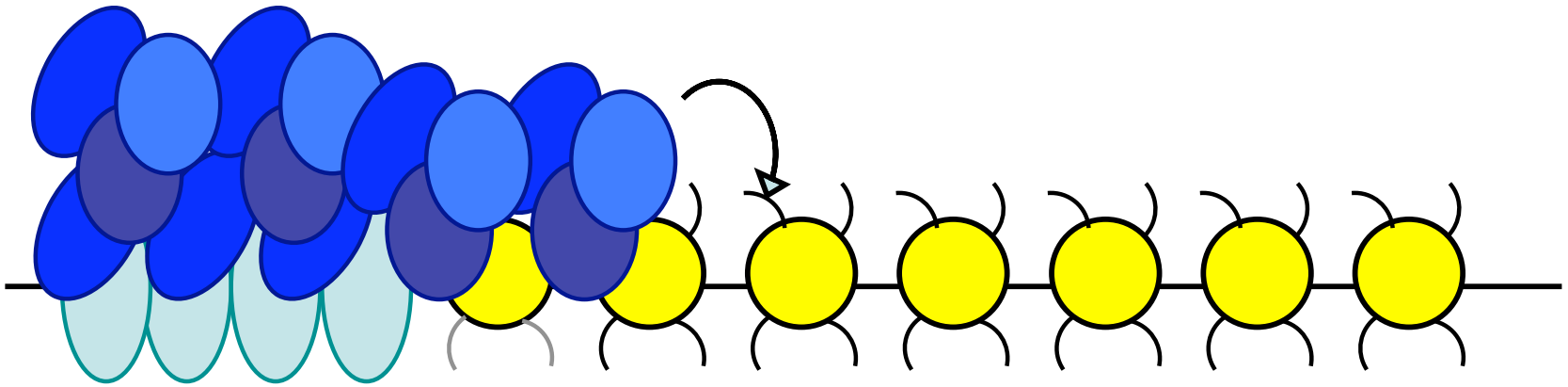
NAD$^+$-dependent histone deacetylase

bind to nucleosome (unacetylated histone tails)

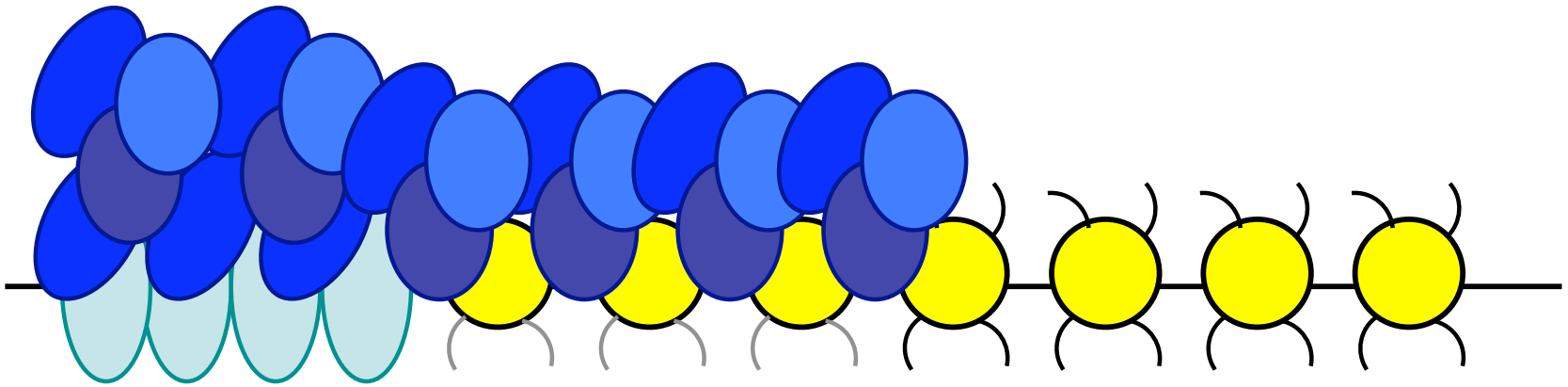
Building Silent Chromatin



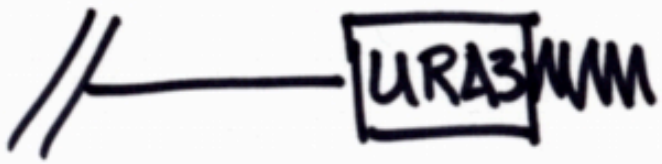
Building Silent Chromatin



Building Silent Chromatin



Screen for Disruptors of Telomeric Silencing

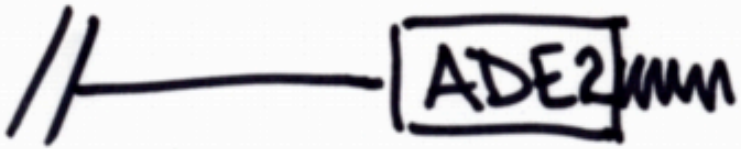


WT

ura⁻

DOT(OE)

Ura⁺



red/white sectored colonies

White

The DOT genes

- ***SIR4*** **Silencing protein**
- ***DOT1*** **Histone H3 methylase**
- ***TLC1*** **Telomerase RNA**
- ***ASF1*** **Chromatin assembly protein**
- ***DNA2*** **DNA replication protein**
- ***DOT4*** **Ubiquitin Hydrolase**
- ***DOT5*** **Nuclear thiol peroxidase(?)**
- ***DOT6*** **putative Myb-DNA binding domain**
- ***IFH1*** **ribosomal RNA expression(?)**
- ***RPL32*** **ribosomal protein**

Fred

van Leeuwen

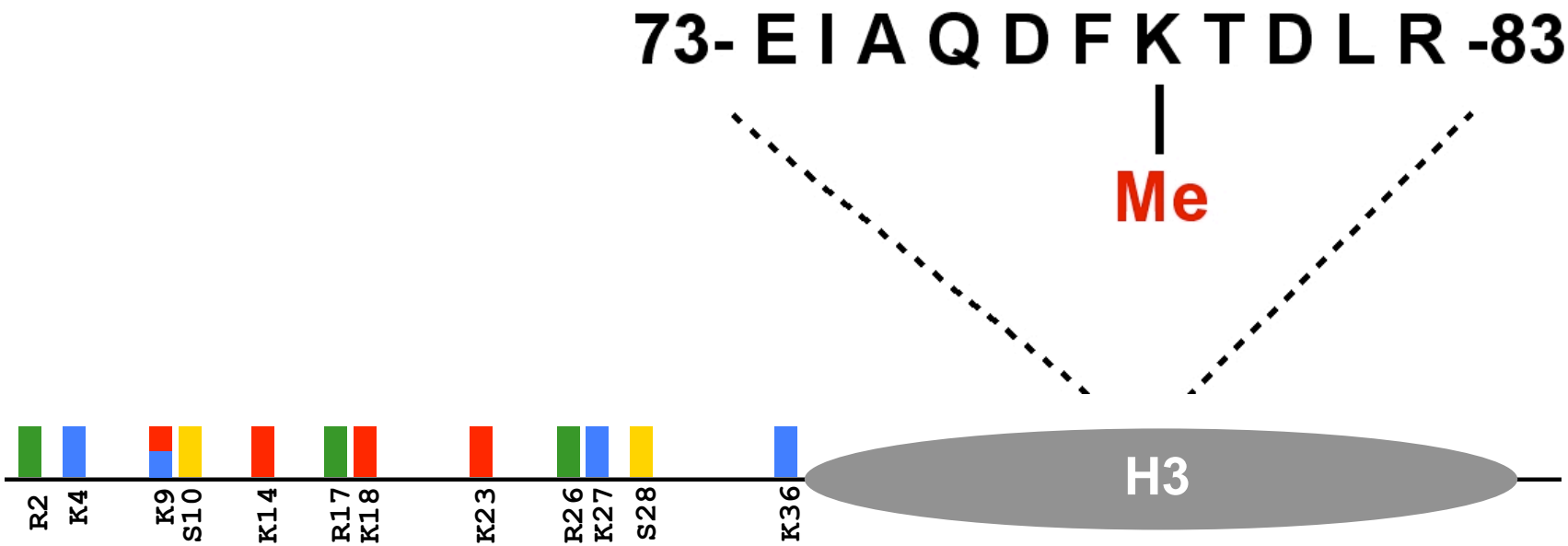
The DOT genes

- *SIR4* Silencing protein
- ***DOT1*** **Histone H3 methylase**
- *TLC1* Telomerase RNA
- *ASF1* Chromatin assembly protein
- *DNA2* DNA replication protein
- *DOT4* Ubiquitin Hydrolase
- *DOT5* Nuclear thiol peroxidase(?)
- *DOT6* putative Myb-DNA binding domain
- *IFH1* ribosomal RNA expression(?)
- *RPL32* ribosomal protein

DOT1 phenotypes

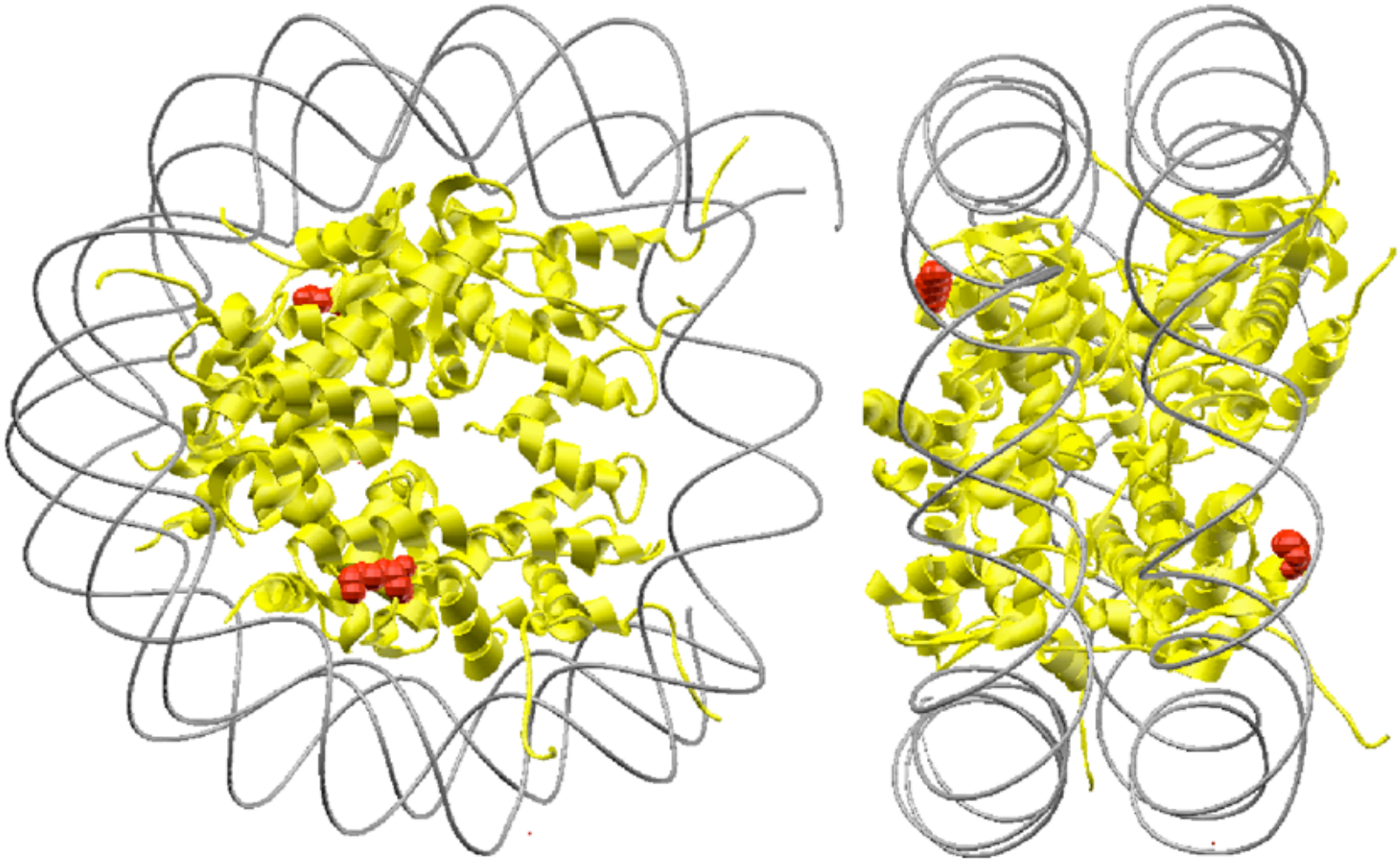
- **Overexpression**
  - **Loss of silencing at telomeres & *HM***
  - **Modest decrease of silencing at rDNA**
- ***dot1Δ***
  - **Decreased silencing at telomeres & *HM* loci**
  - **Little or no effect at rDNA**
  - **Reduced levels of Y' RNA [Hughes et al. (2000)]**
  - **Diffuse staining of Sir3 along mitotic chromosomes [San-Segundo & Roeder (2000)]**

Dot1 methylates histone H3 on Lys79



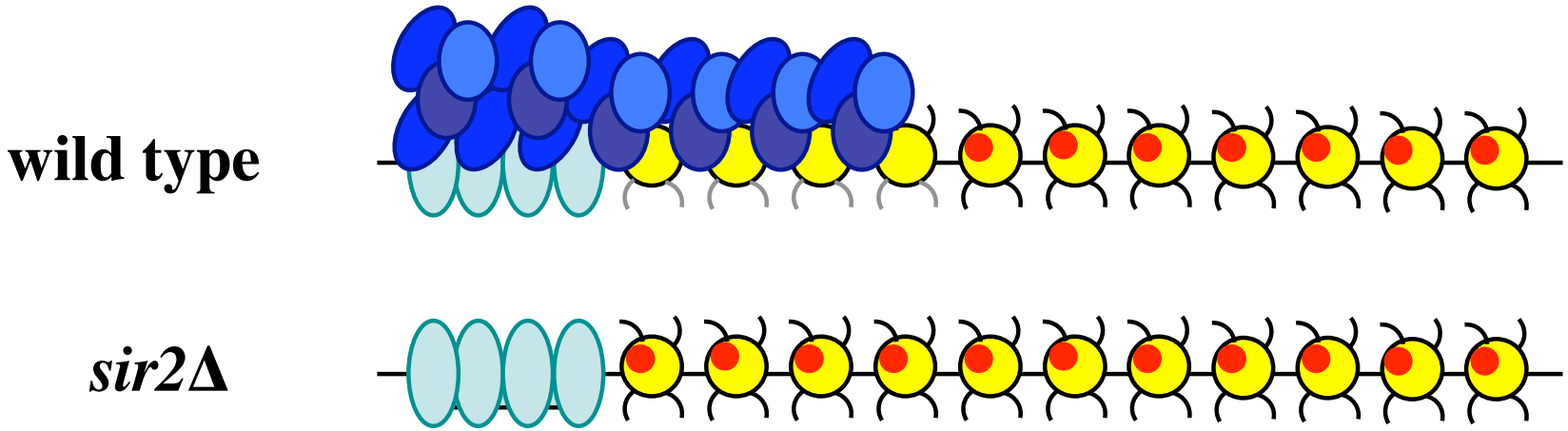
Dot1 methylates histone H3 only in chromatin

Histone H3-Lys79 is located on the top and bottom of the nucleosome core



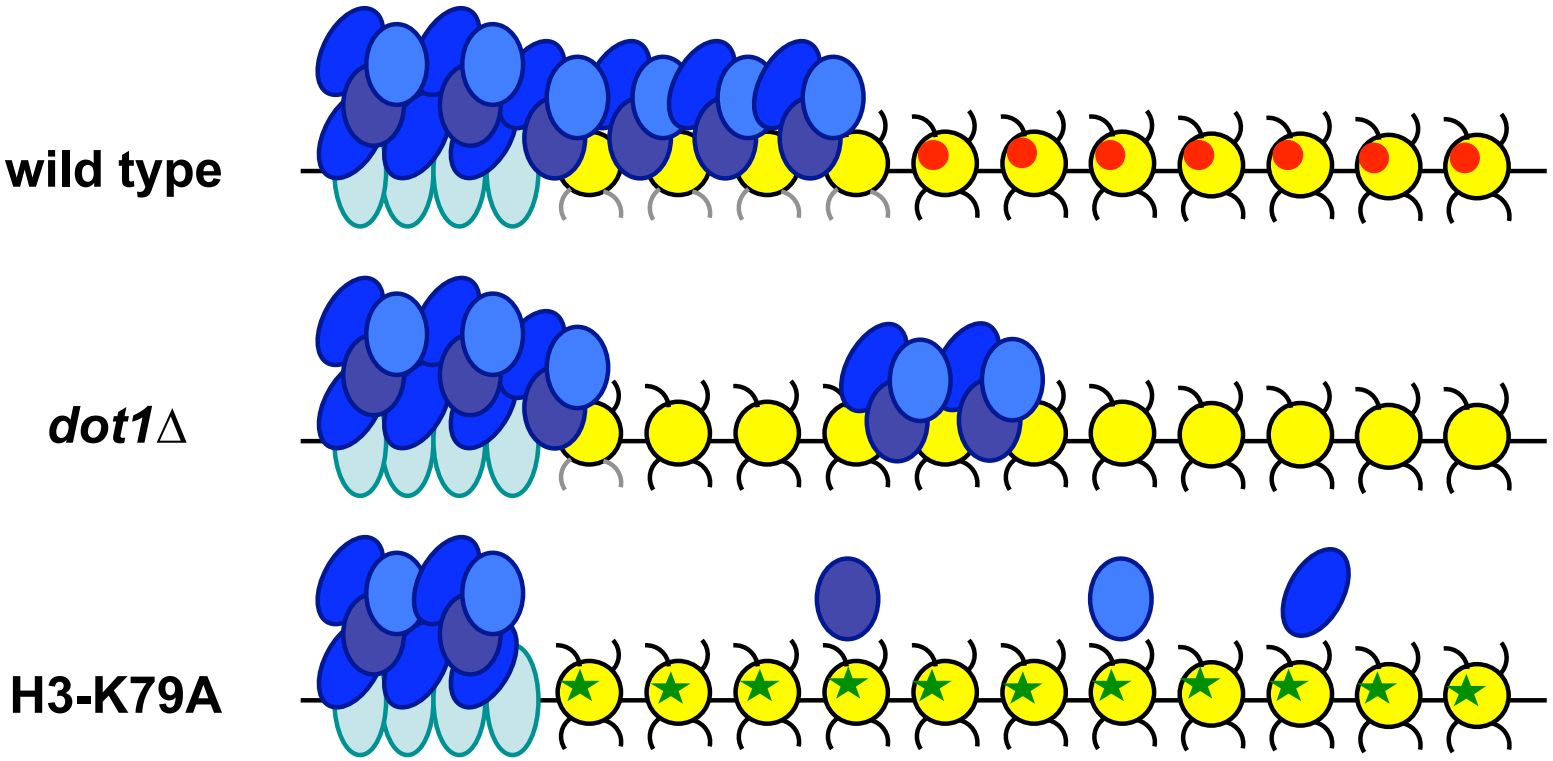
K. Luger EMBO 20:5207 (2001)

Methylated histone H3-K79 is a mark of euchromatin

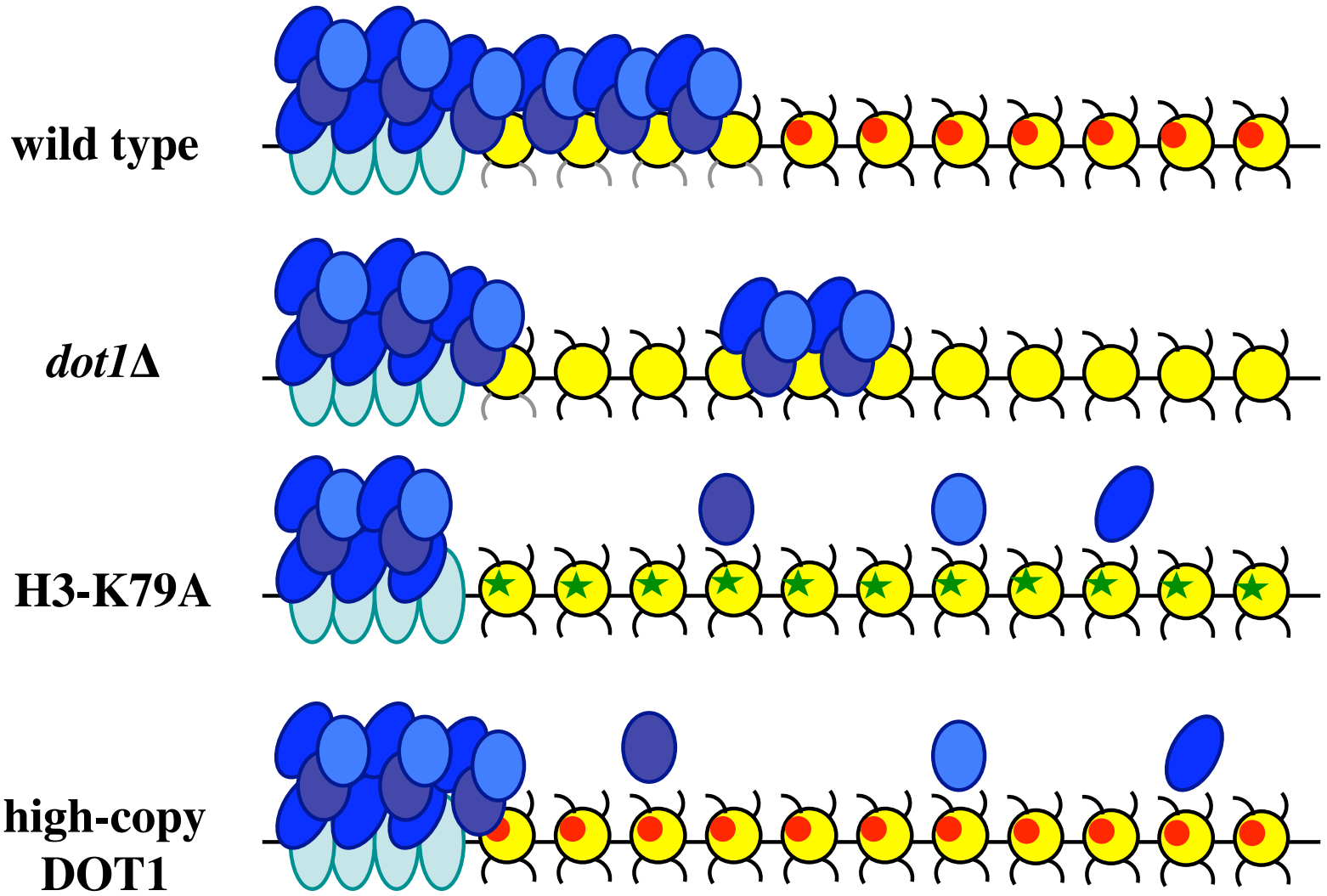


- ♣90% of total chromatin is methylated
- ♣Silent loci are hypomethylated at histone H3-K79
- ♣ Silent chromatin prevents methylation of H3-K79 by Dot1

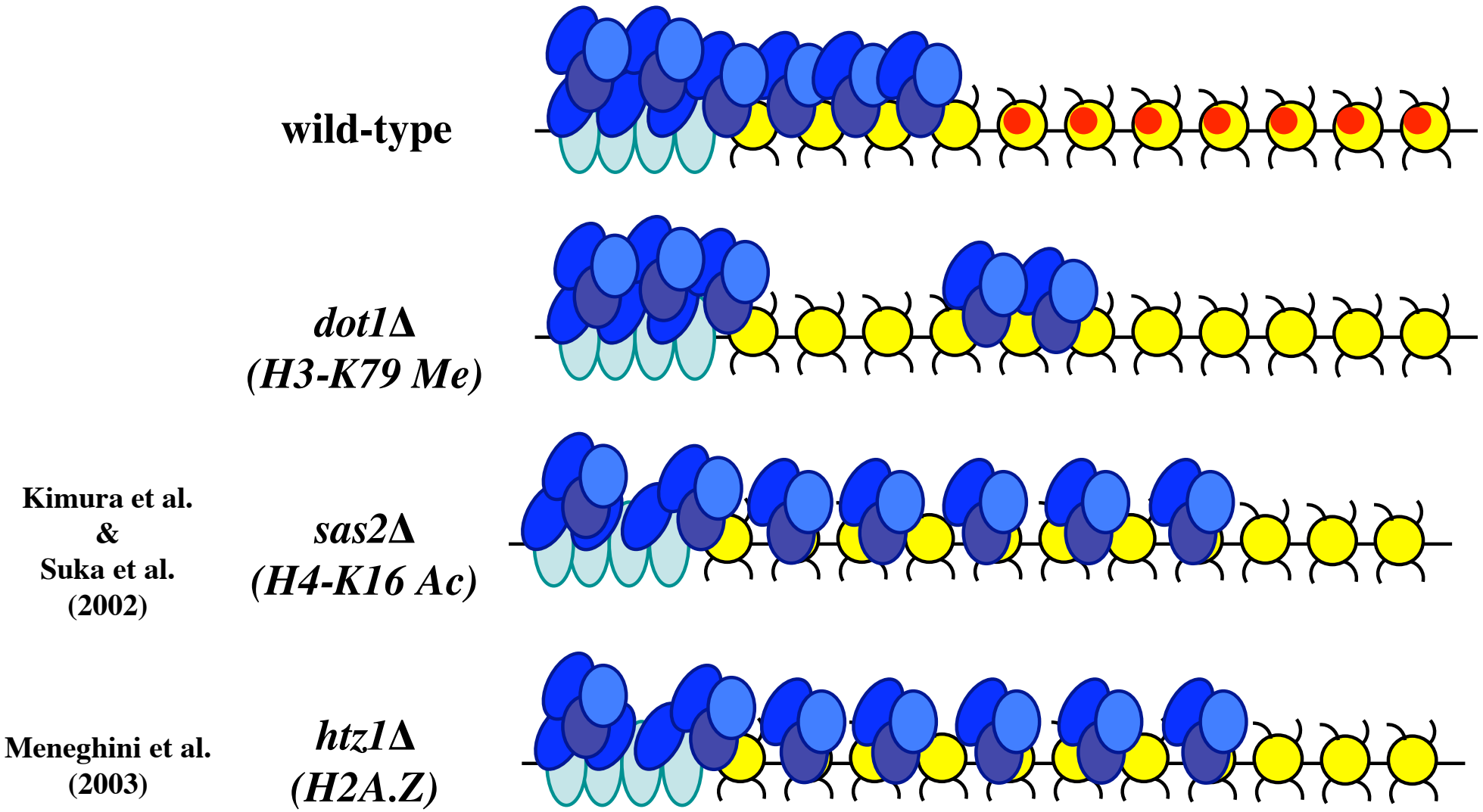
Loss of H3-K79 methylation results in redistribution of Sir proteins



Dot1 reduces promiscuous binding of silencing proteins in euchromatin



Gaining specificity by preventing promiscuity: A common theme?



DOT1 requires RAD6 activity *in vivo*

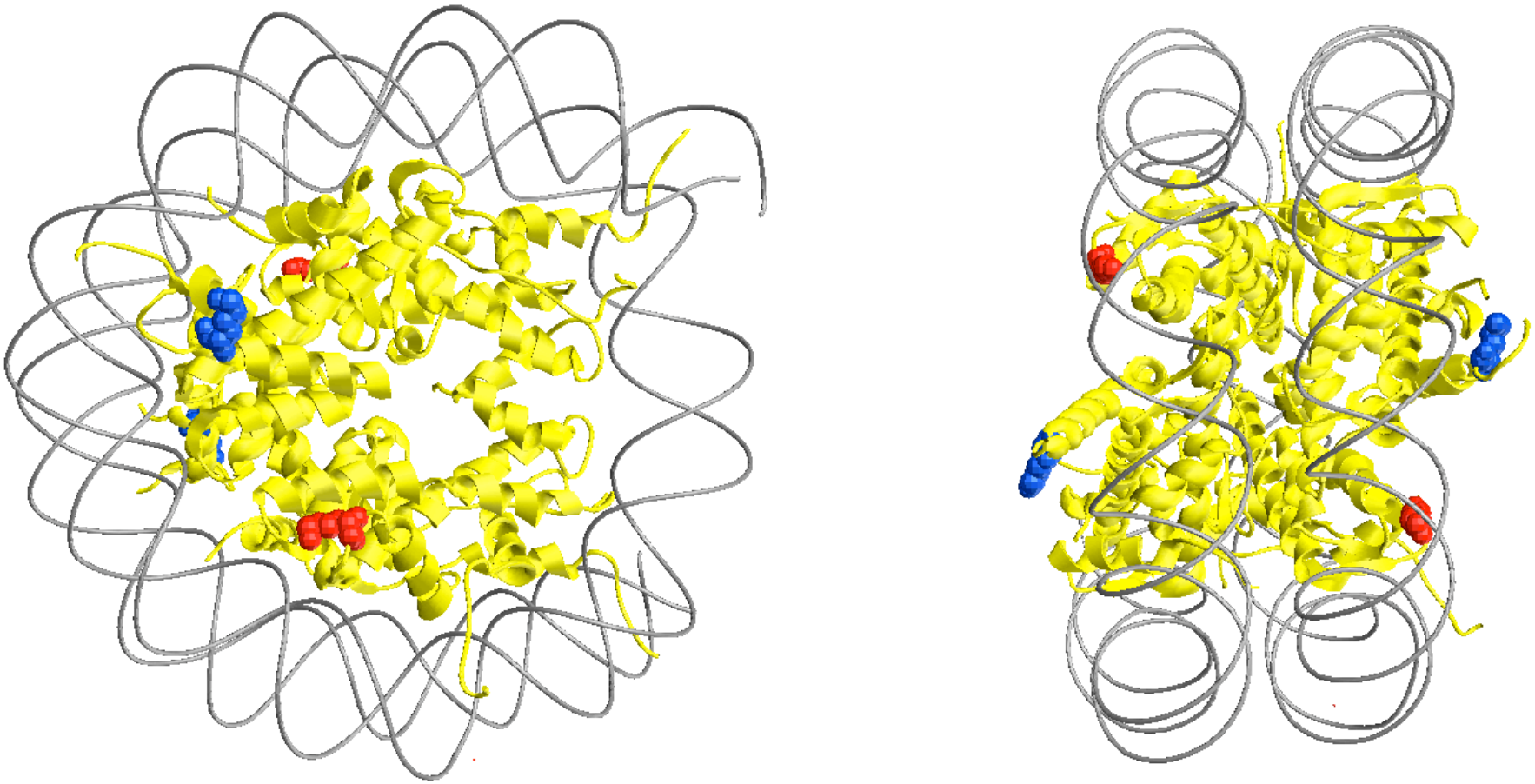
- **RAD6 mono-ubiquitinates K123 of histone H2B**

  [Robzyk et al. (2000)]

  - ♣ **Required for silencing [Huang et al. (1997)]**
  - ♣ **Required for histone H3 methylation of K79**

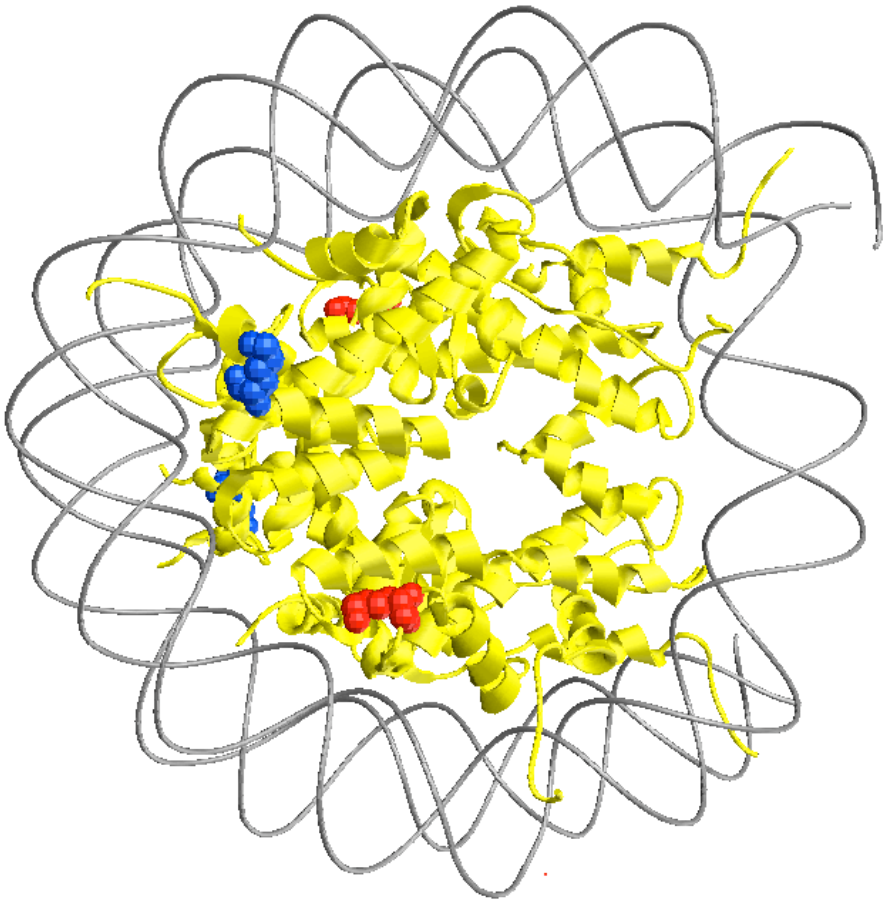
    [Sun & Allis, Ng et al. & Dover et al. (2002)]

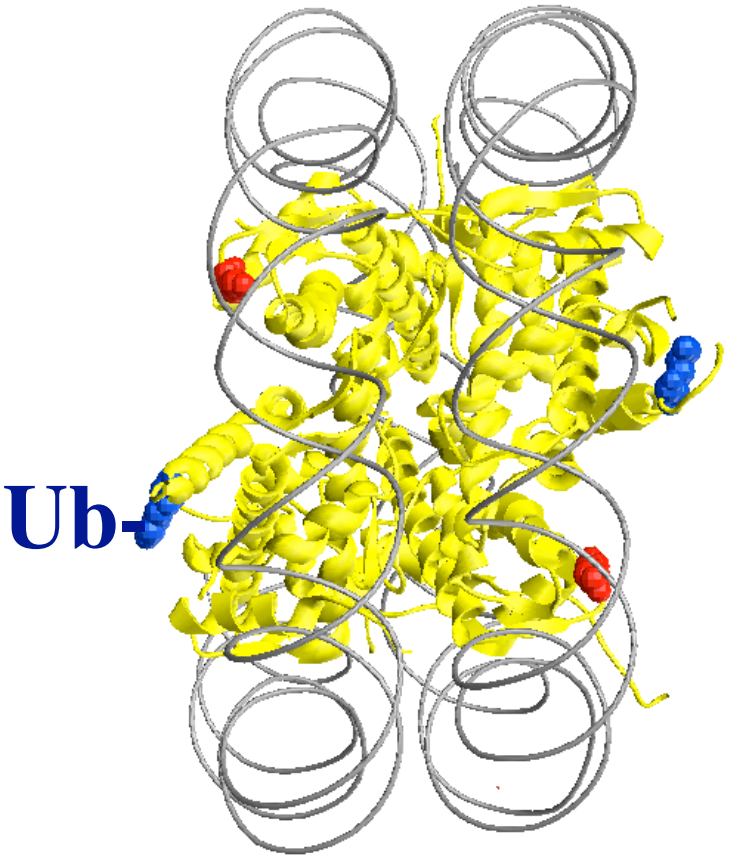
DOT1 needs RAD6 to prevent promiscuous Sir binding



H3-K79 H2B-K123

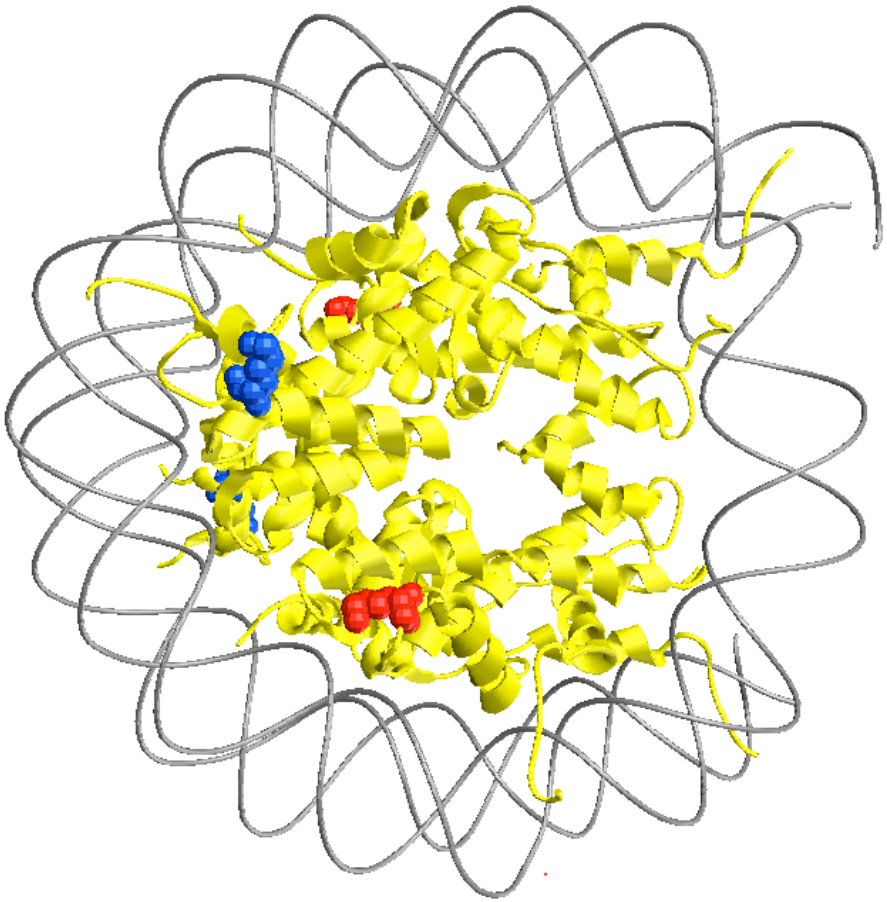
DOT1 needs RAD6 to prevent promiscuous Sir binding

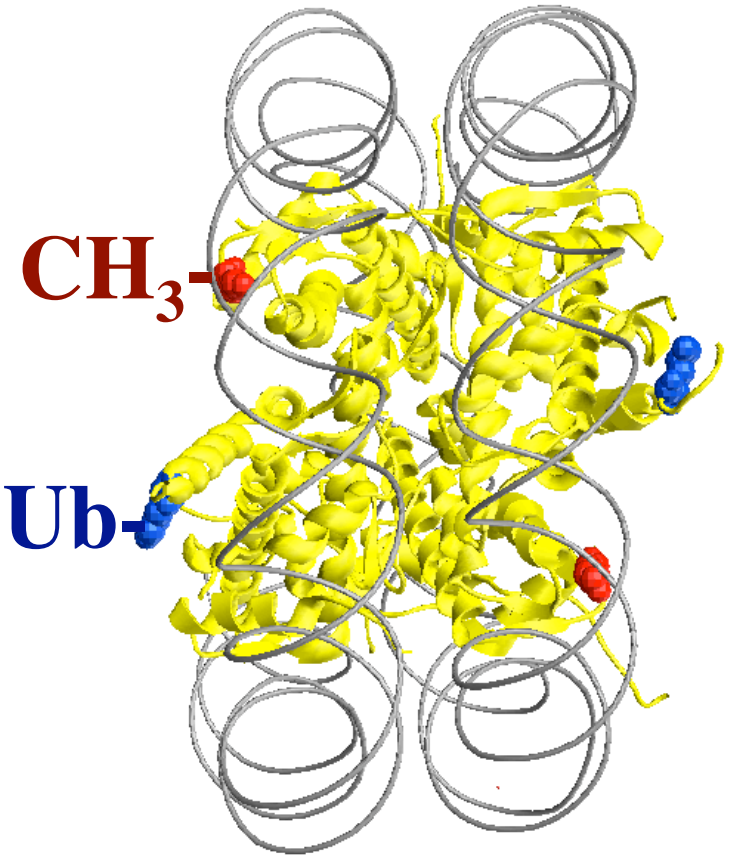




H3-K79 H2B-K123

DOT1 needs RAD6 to prevent promiscuous Sir binding





H3-K79 H2B-K123

A reversible modification can be converted into a permanent "memory mark"

The DOT genes

- *SIR4* Silencing protein
- *DOT1* Histone H3 methylase
- *TLC1* Telomerase RNA
- *ASF1* Chromatin assembly protein
- *DNA2* DNA replication protein
- ***DOT4*** **Ubiquitin Hydrolase**
- *DOT5* Nuclear thiol peroxidase(?)
- *DOT6* putative Myb-DNA binding domain
- *IFH1* ribosomal RNA expression(?)
- *RPL32* ribosomal protein

DOT4 (*UBP10*)

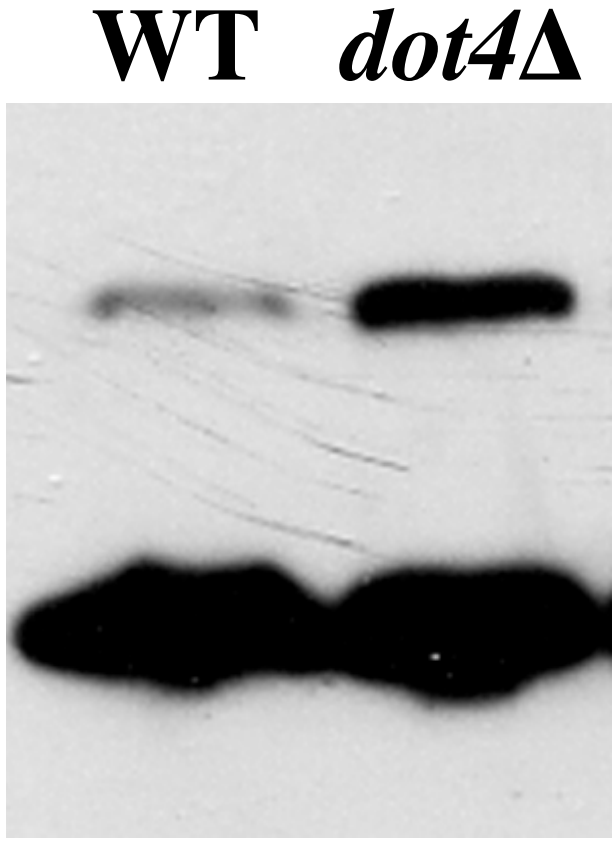
[MCB 19:6608 (1999)]

- **Ubiquitin Hydrolase**
- **Binds to Sir4p**

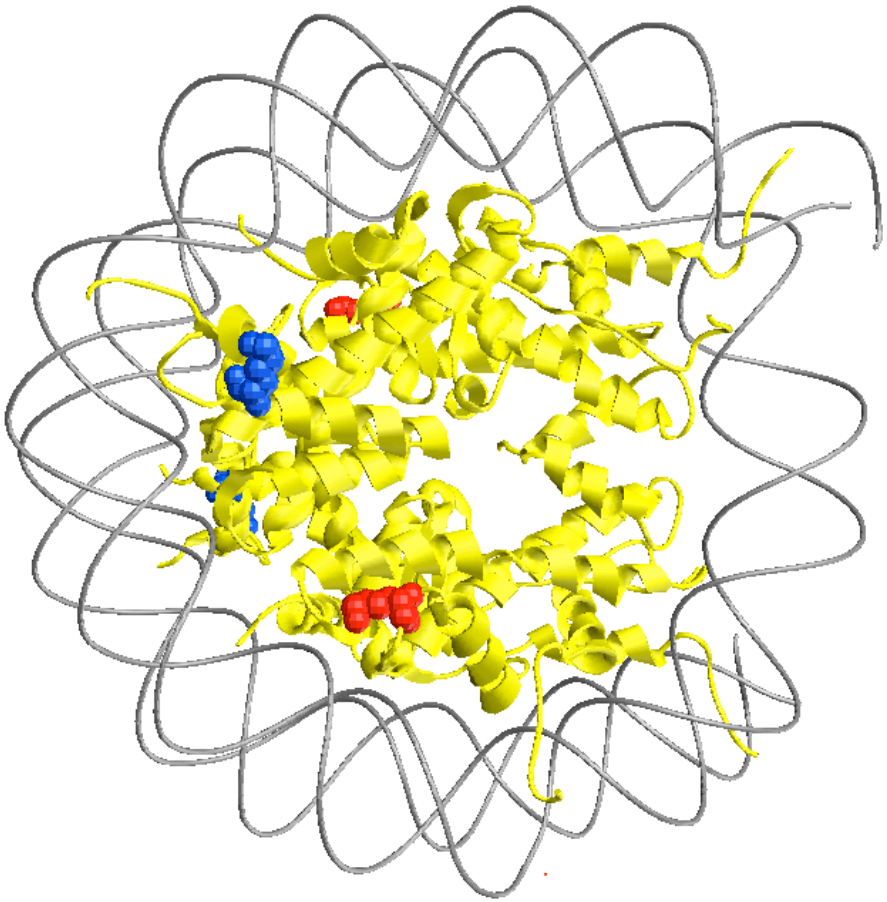
DOT4 keeps nucleosomes from receiving a permanent "active" mark

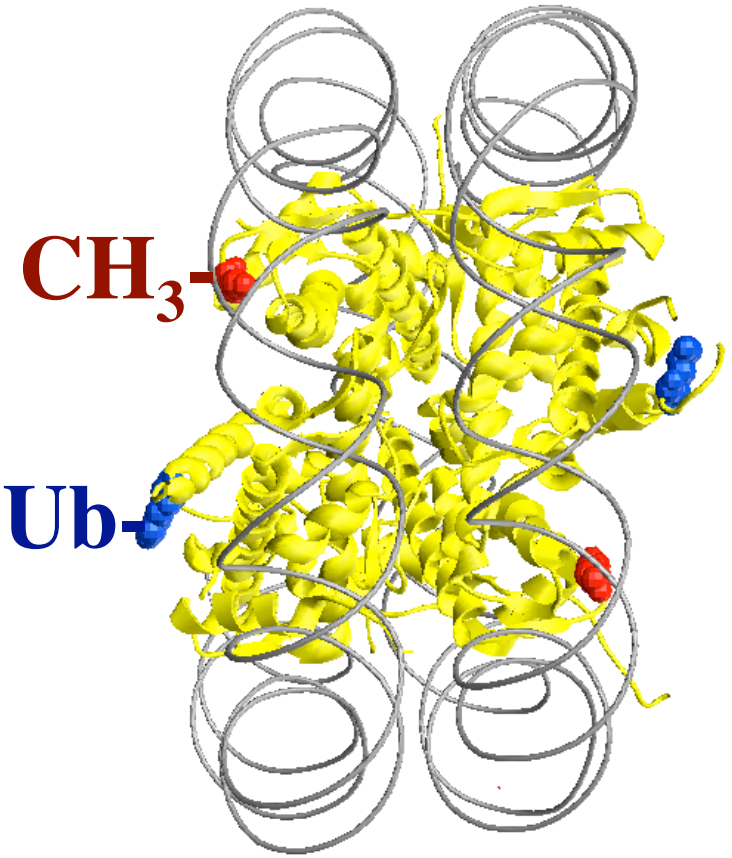
- Ubiquitin Hydrolase
- Binds to Sir4p
- Antagonizes Rad6p ubiquitination of histone H2B

(Rich Gardner)



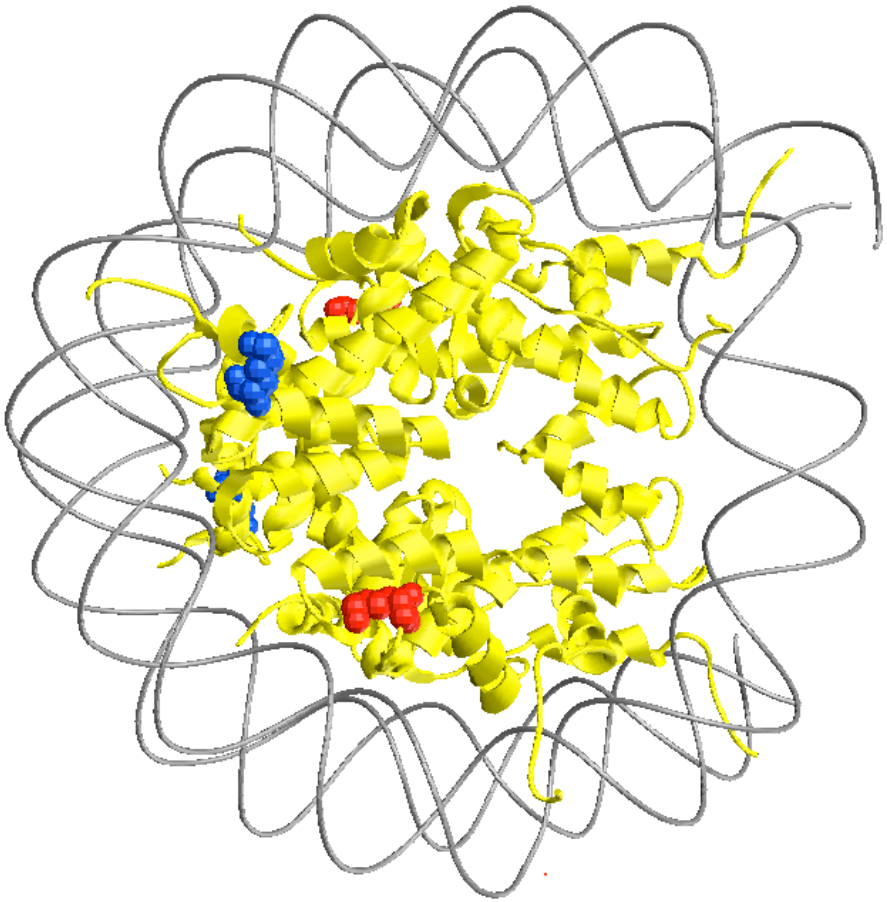
DOT4 keeps nucleosomes from receiving a permanent “active” mark

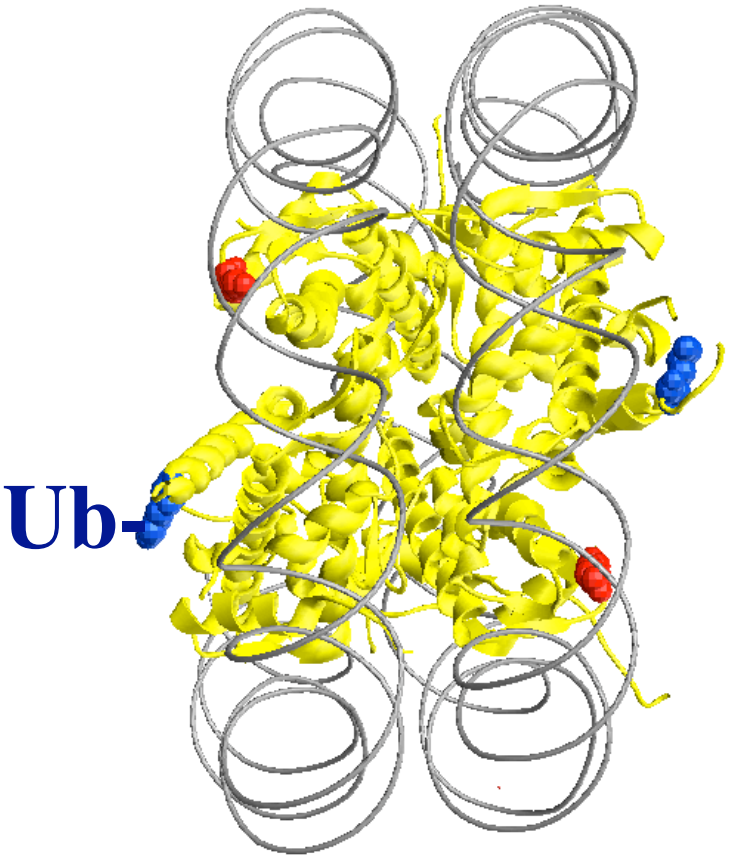




H3-K79 H2B-K123

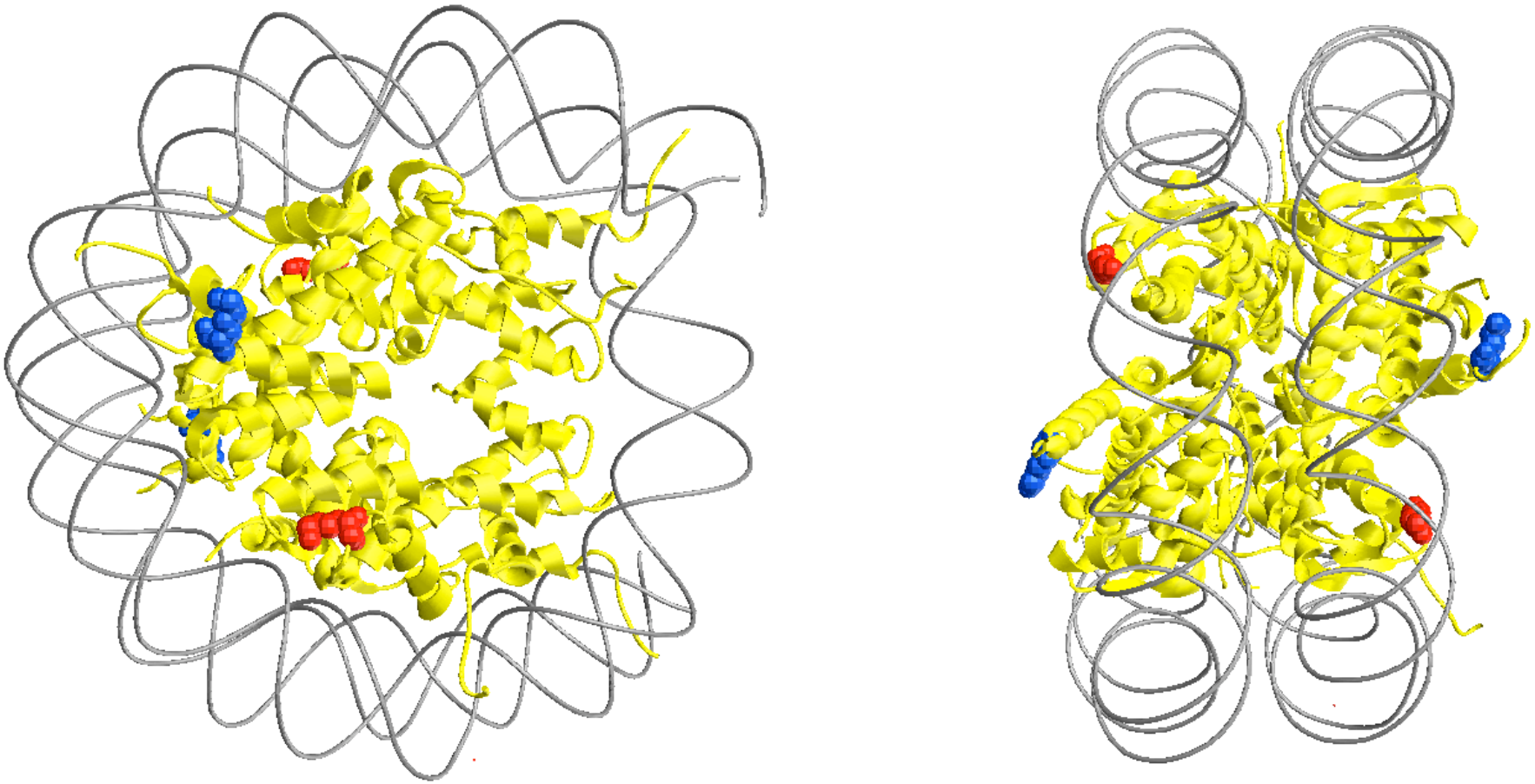
DOT4 keeps nucleosomes from receiving a permanent “active” mark





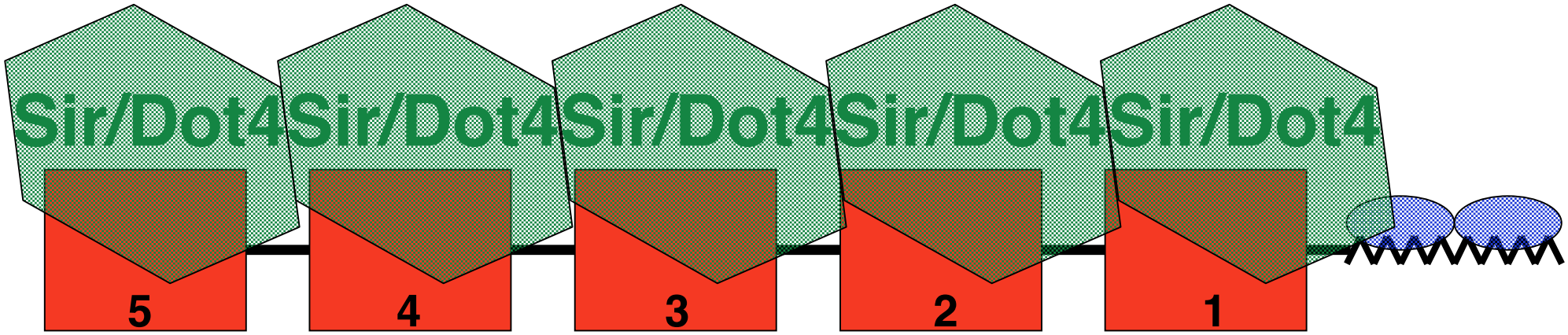
H3-K79 H2B-K123

DOT4 keeps nucleosomes from receiving a permanent “active” mark

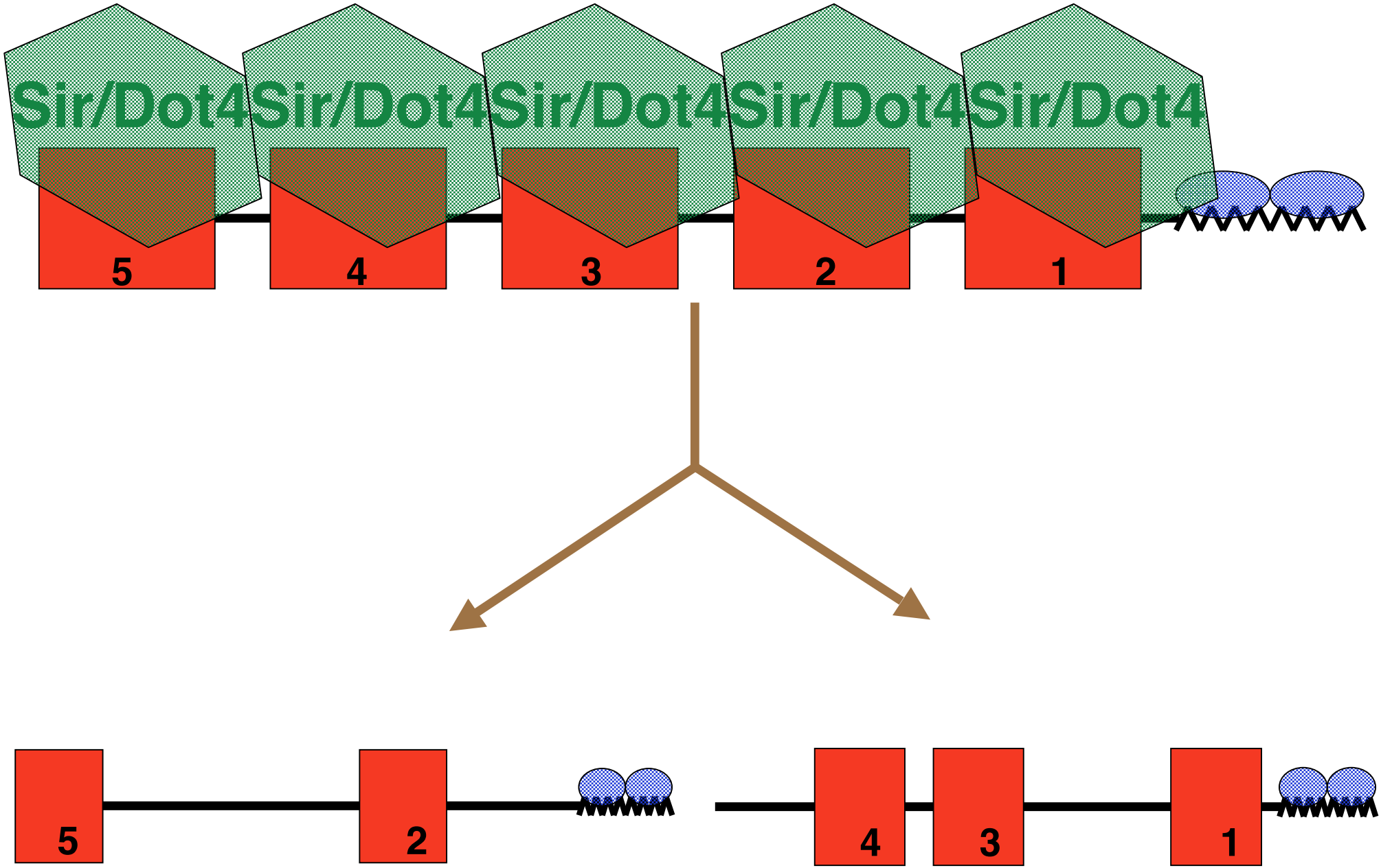


H3-K79 H2B-K123

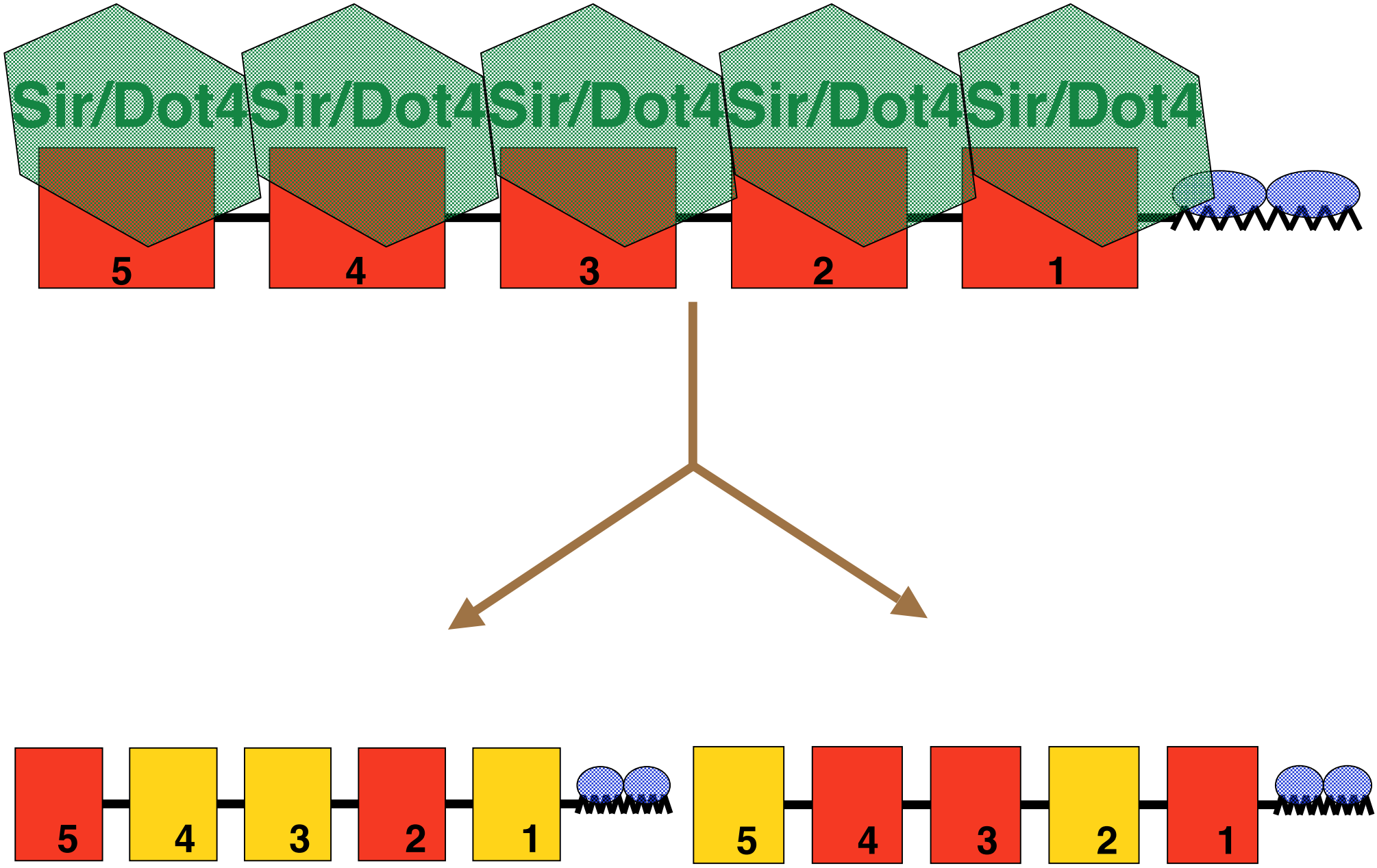
Propagating Silent Chromatin



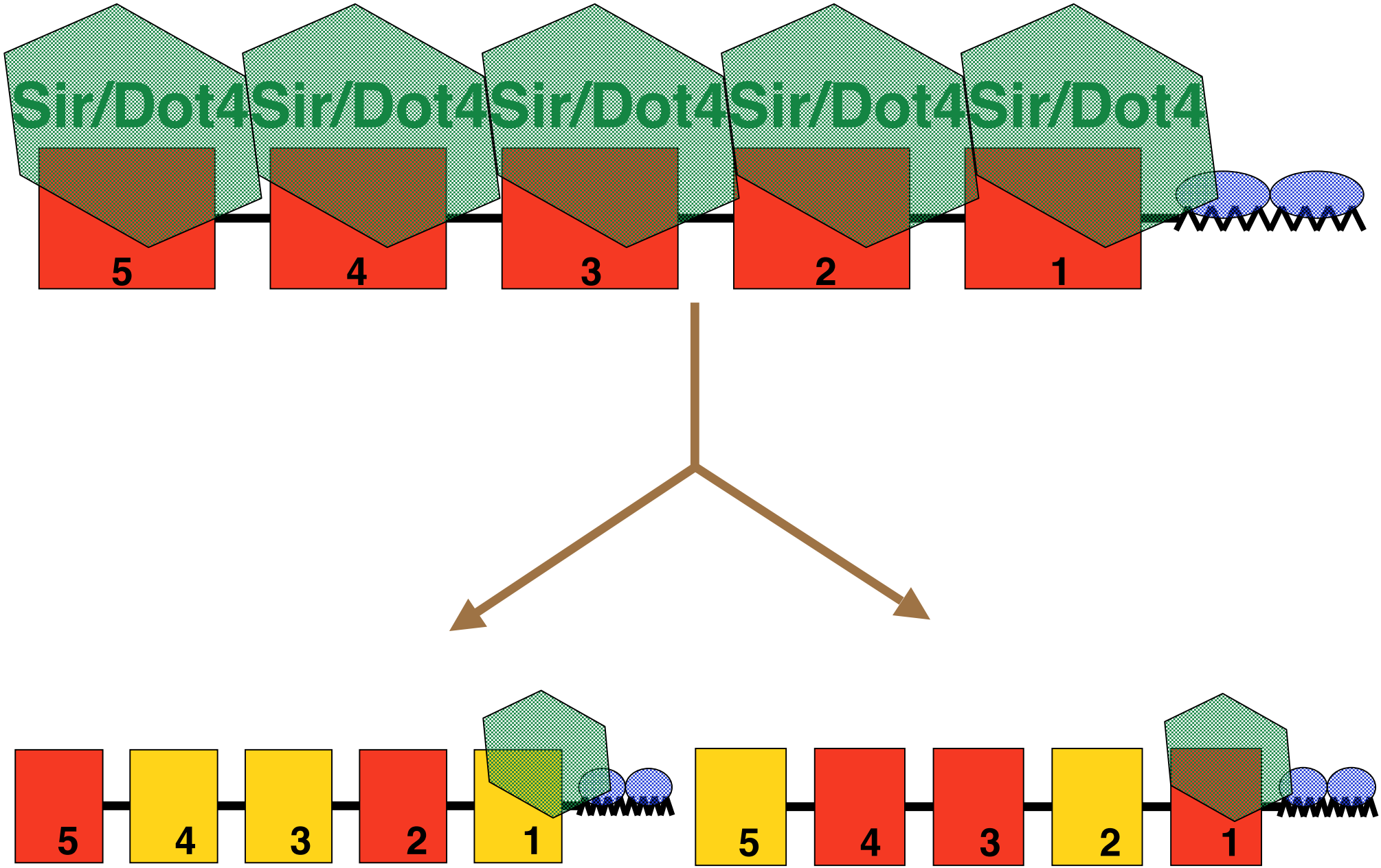
Propagating Silent Chromatin



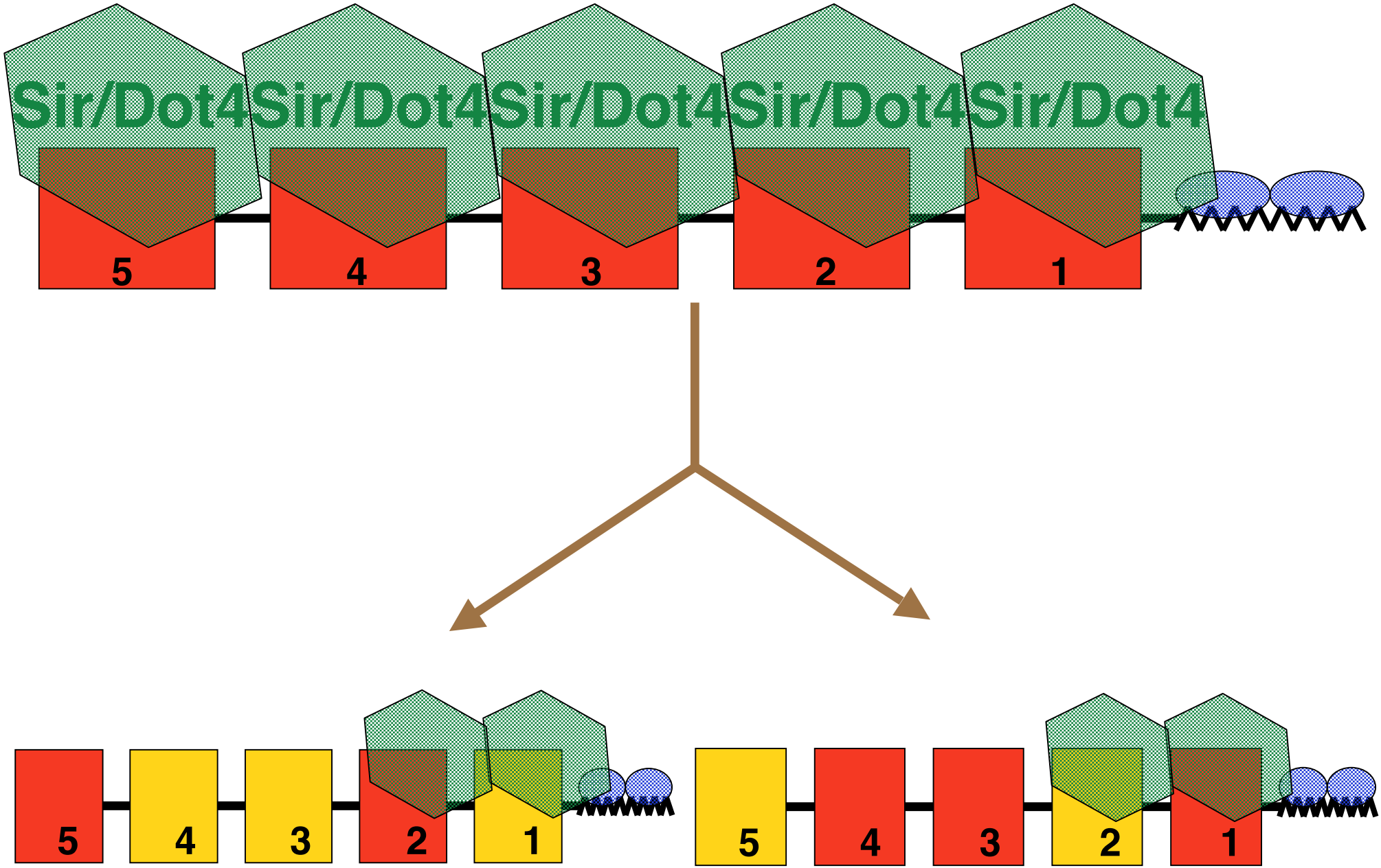
Propagating Silent Chromatin



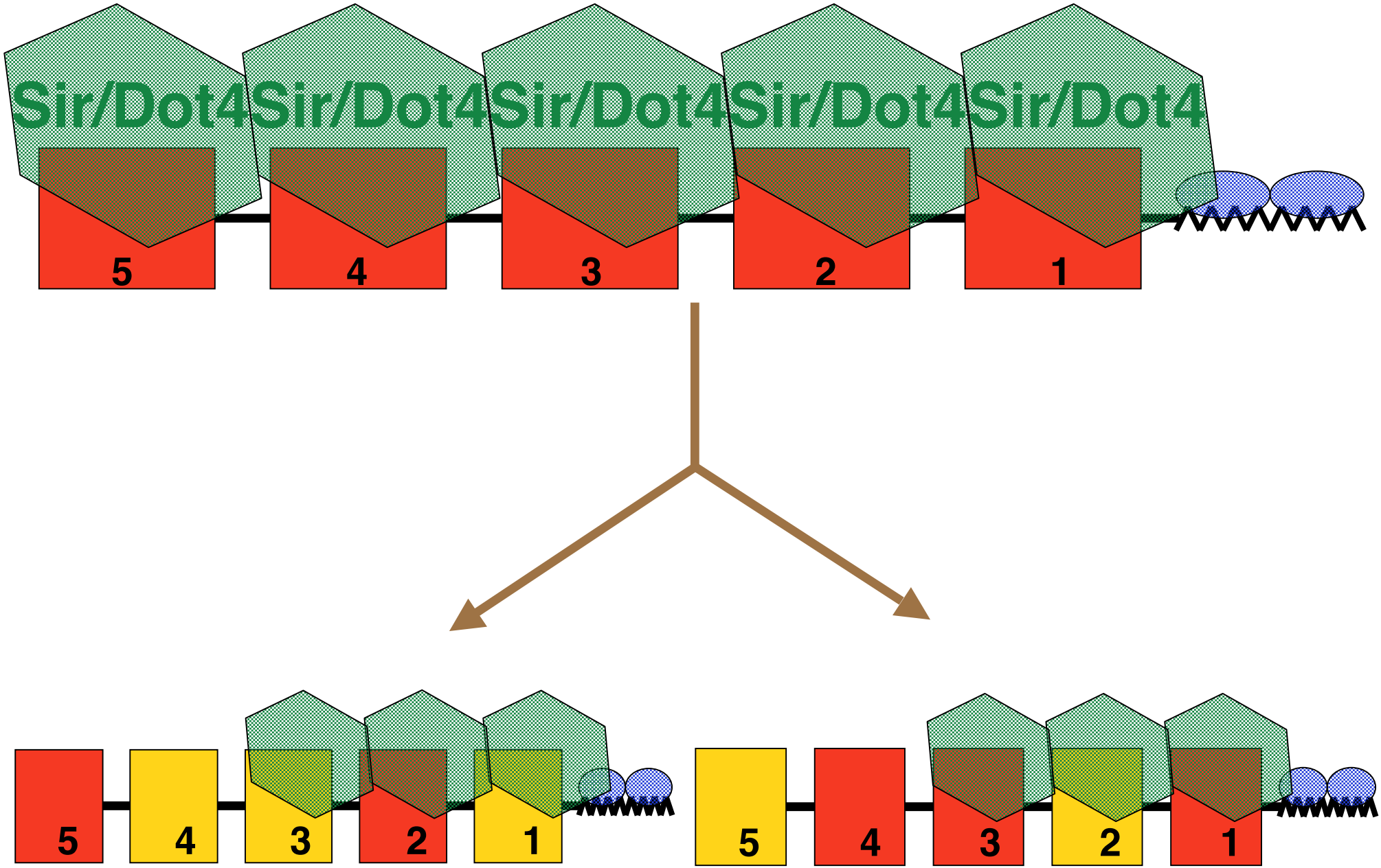
Propagating Silent Chromatin



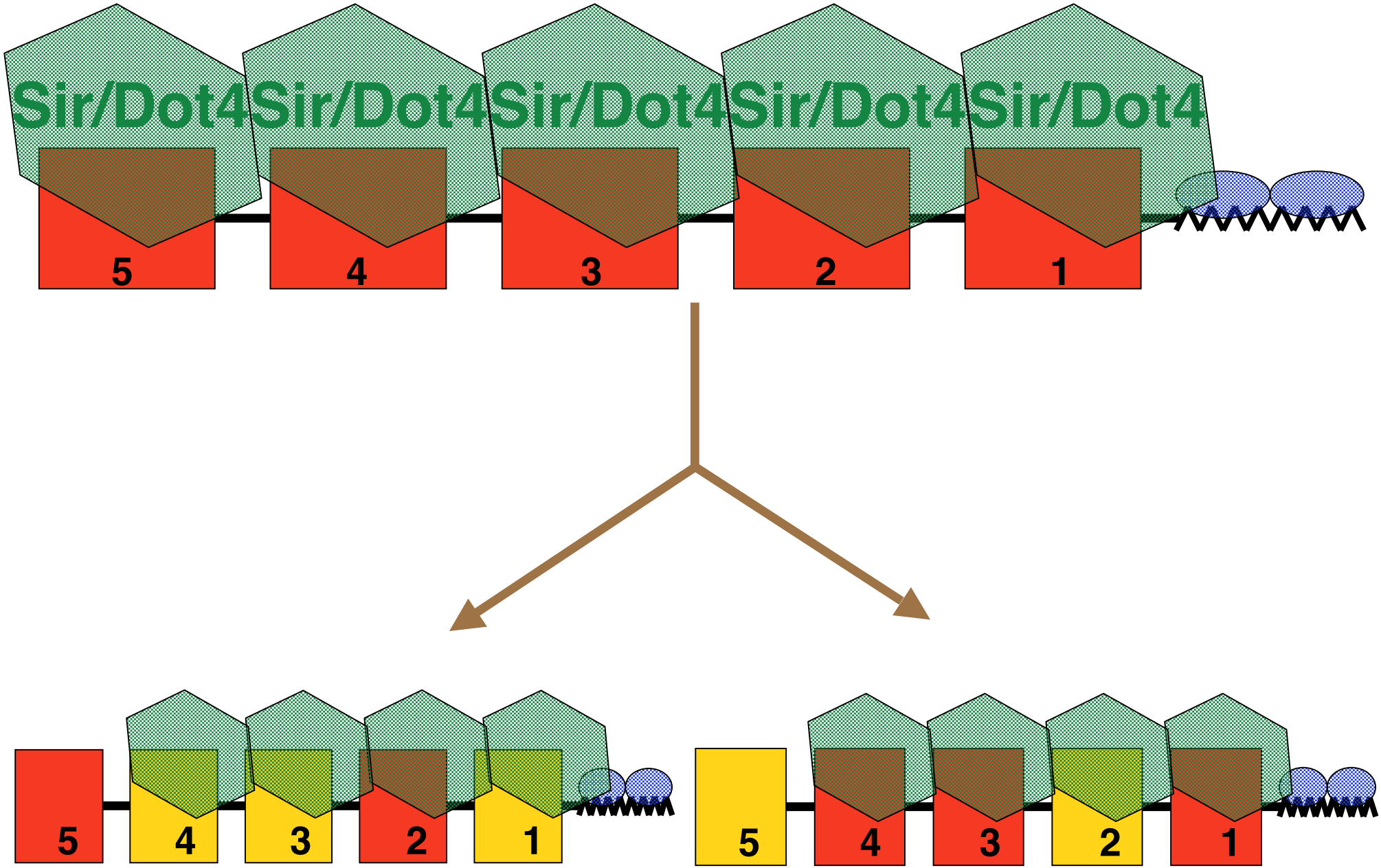
Propagating Silent Chromatin



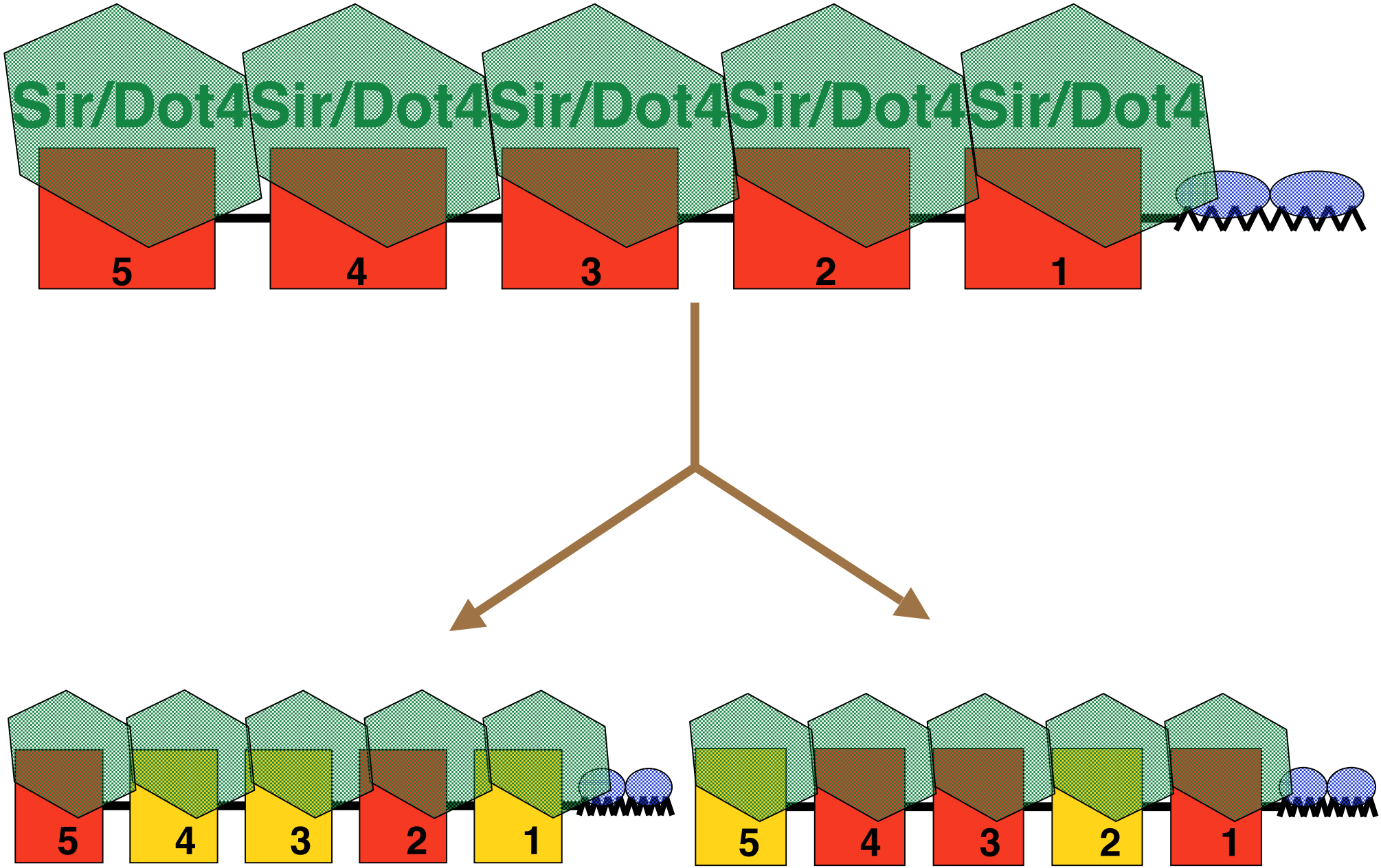
Propagating Silent Chromatin



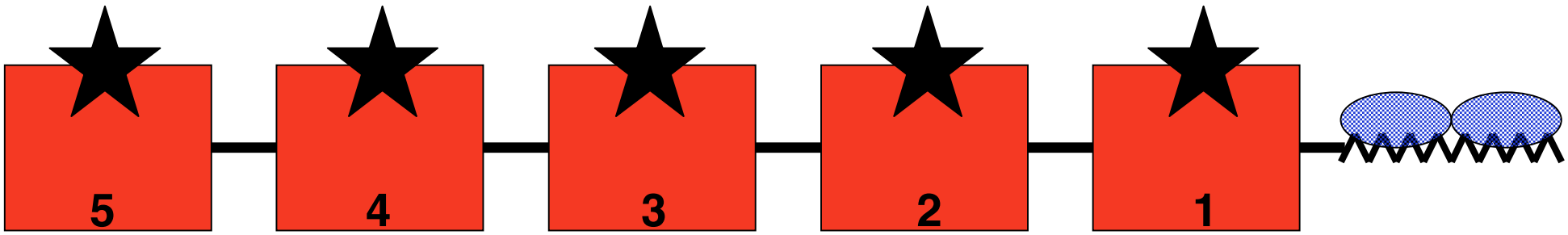
Propagating Silent Chromatin



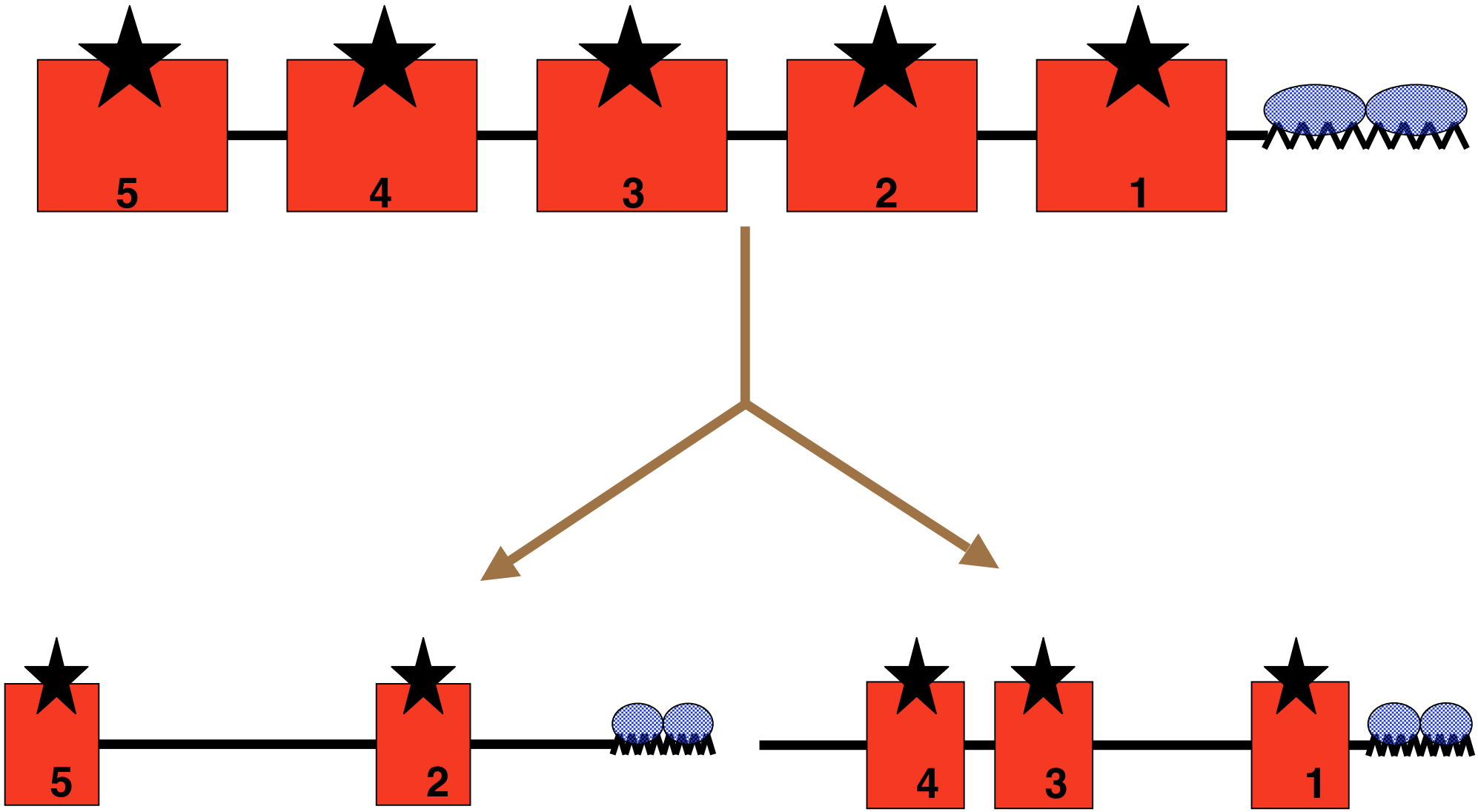
Propagating Silent Chromatin



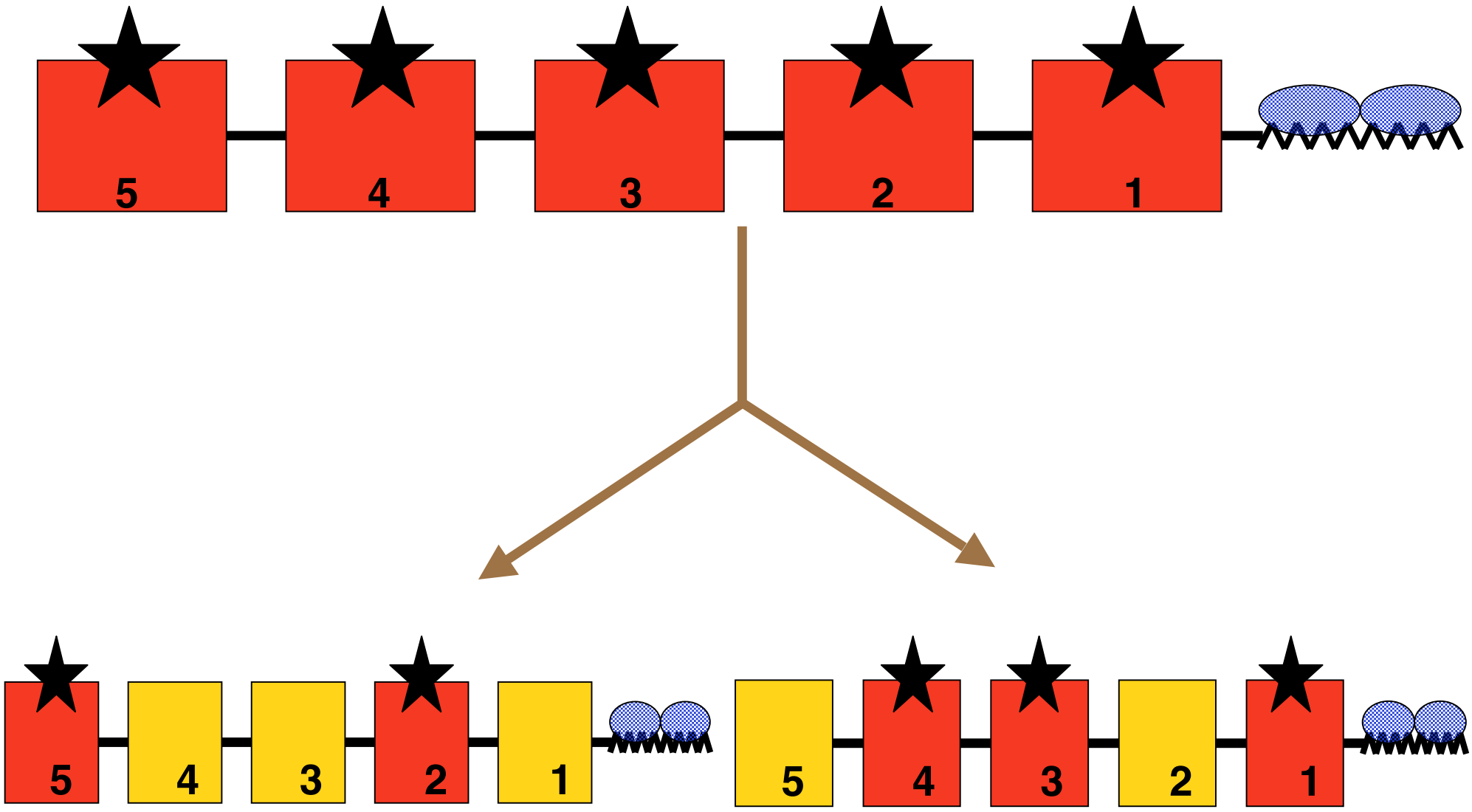
Propagating Active Chromatin



Propagating Active Chromatin



Propagating Active Chromatin



Propagating Active Chromatin