Lecture 6

Chromosome Structure and Function in Mitosis

Outline:

Chromosome Organization and Function in Interphase

- Chromatin effects on replication
- Effects of nuclear organization on gene expression

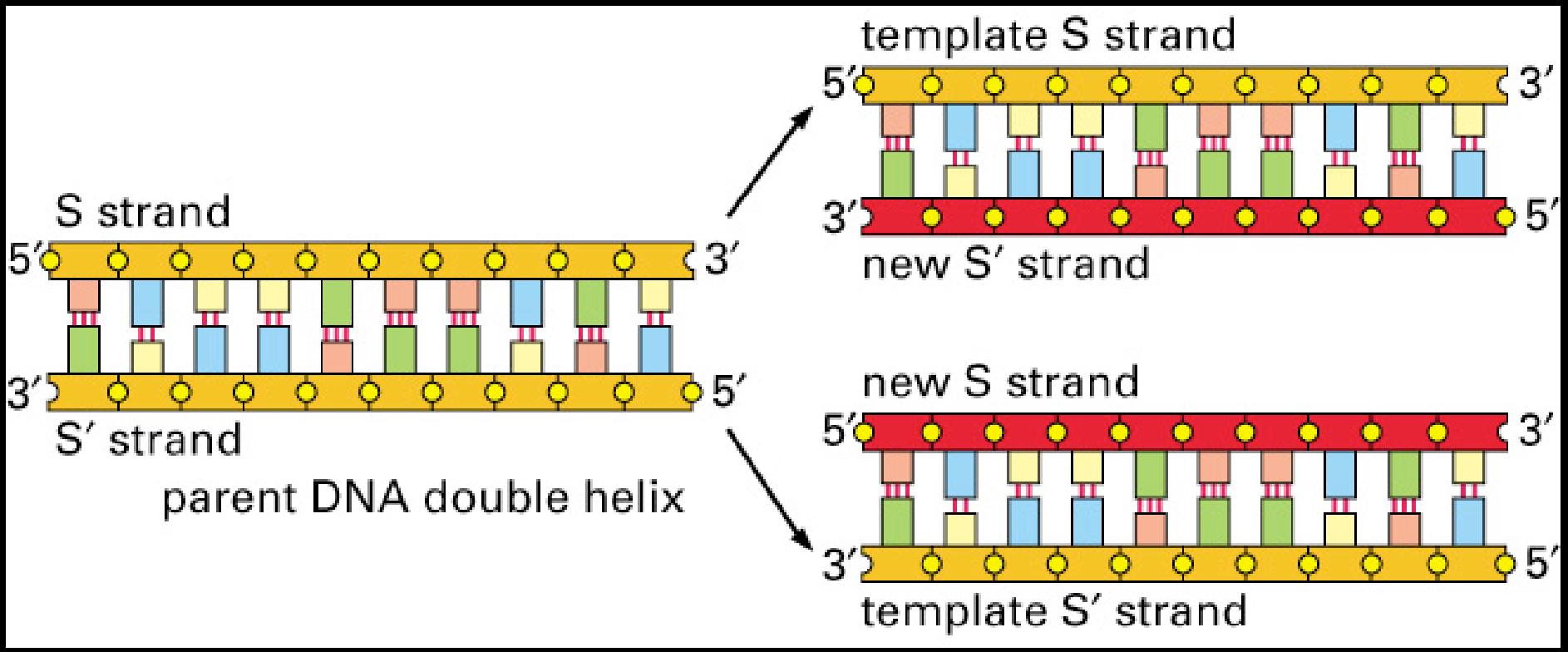
Chromosomes in Mitosis

- SMC proteins:Condensins and Cohesins
- Centromeres and Kinetochores

Paper: Loss of the Suv39h Histone Methyltransferases Impairs Mammalian Heterochromatin and Genome Stability

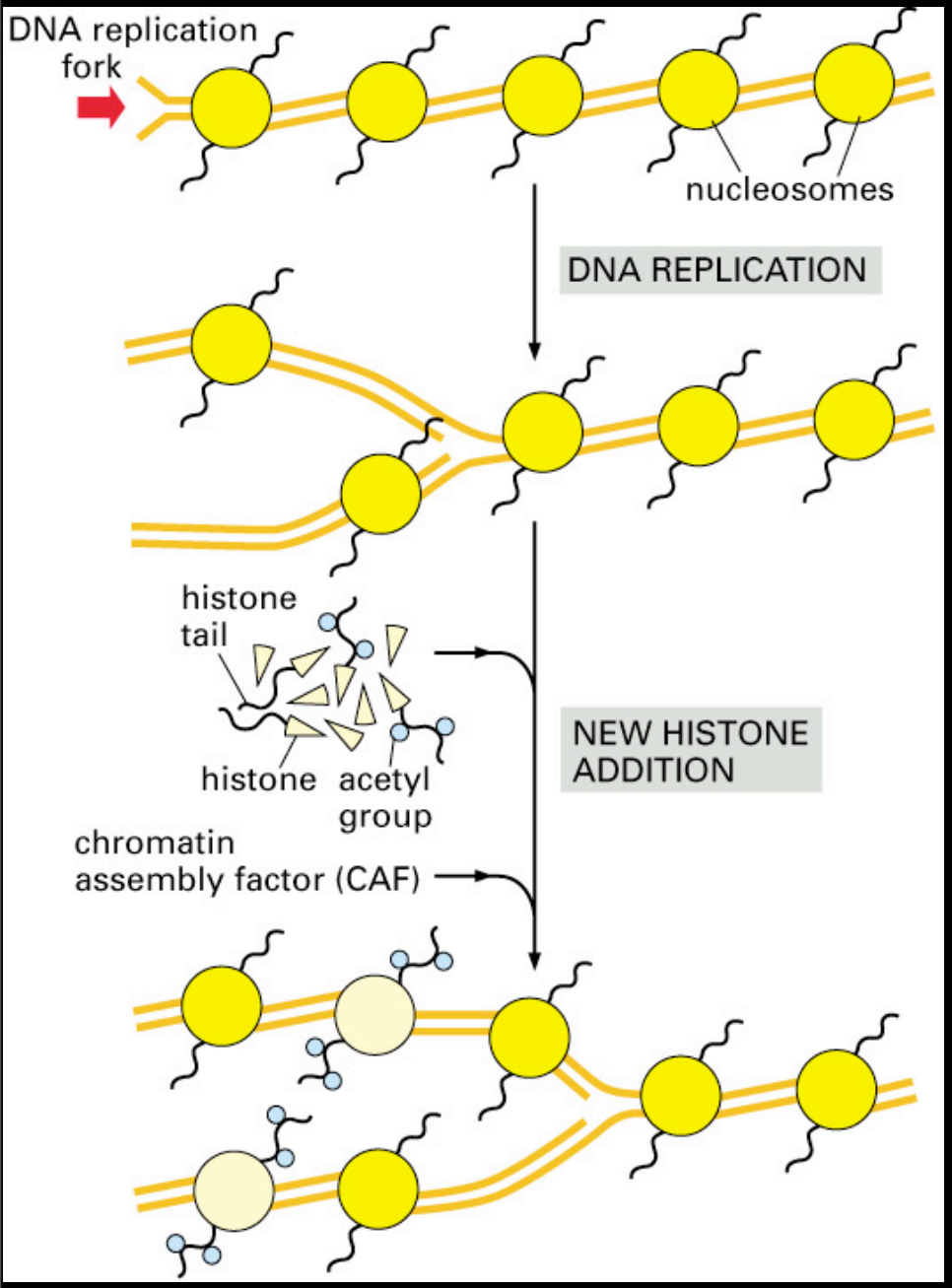
Chromatin Structure and DNA Replication

DNA replication is ‘simple’....



...though the regulation of molecules (e.g. DNA polymerases, helicases) is not...

Chromatin is also 'Replicated' Semi-conservatively



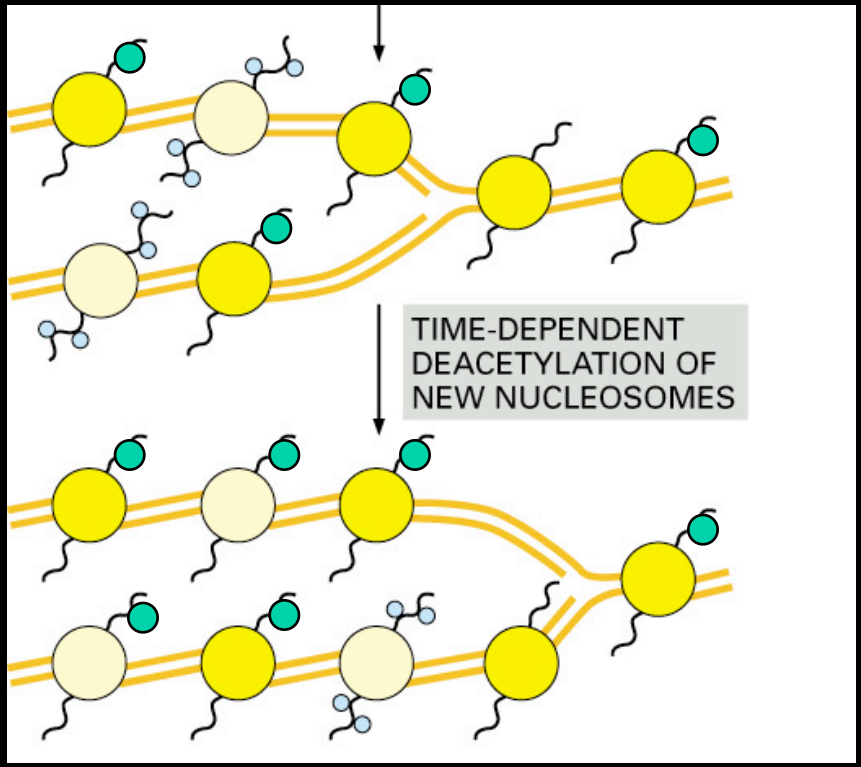
requires

- energy
- remodeling factors (Swi/Snf helicases)
- assembly factors
  - CAFs-canonical histones
  - HIRA-histone variants

Epigenetic Patterns are Reestablished after Replication

Histones contain specific acetylated K residues prior to assembly
removed after assembly

removal required for other modifications of new nucleosomes, eg methylation



- **H3K9me**

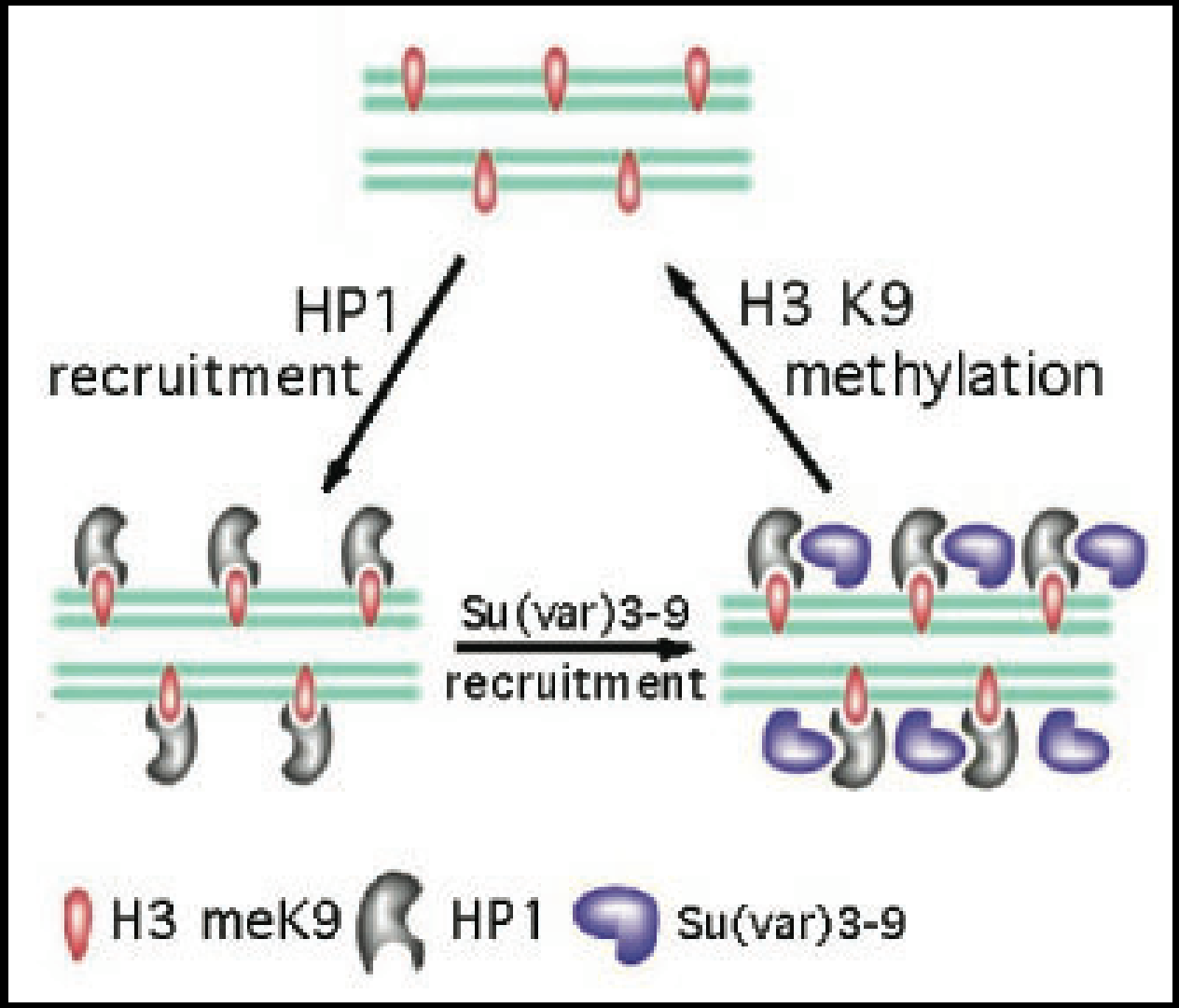
parental nucleosomes and their modifications segregated to daughter strands

propagation of modifications on new nucleosomes based on parental nucleosomes via binding protein / modification enzyme mechanism (e.g. HP1 and Su(var)3-9)

Propagation of a ‘Silent’ Epigenetic State

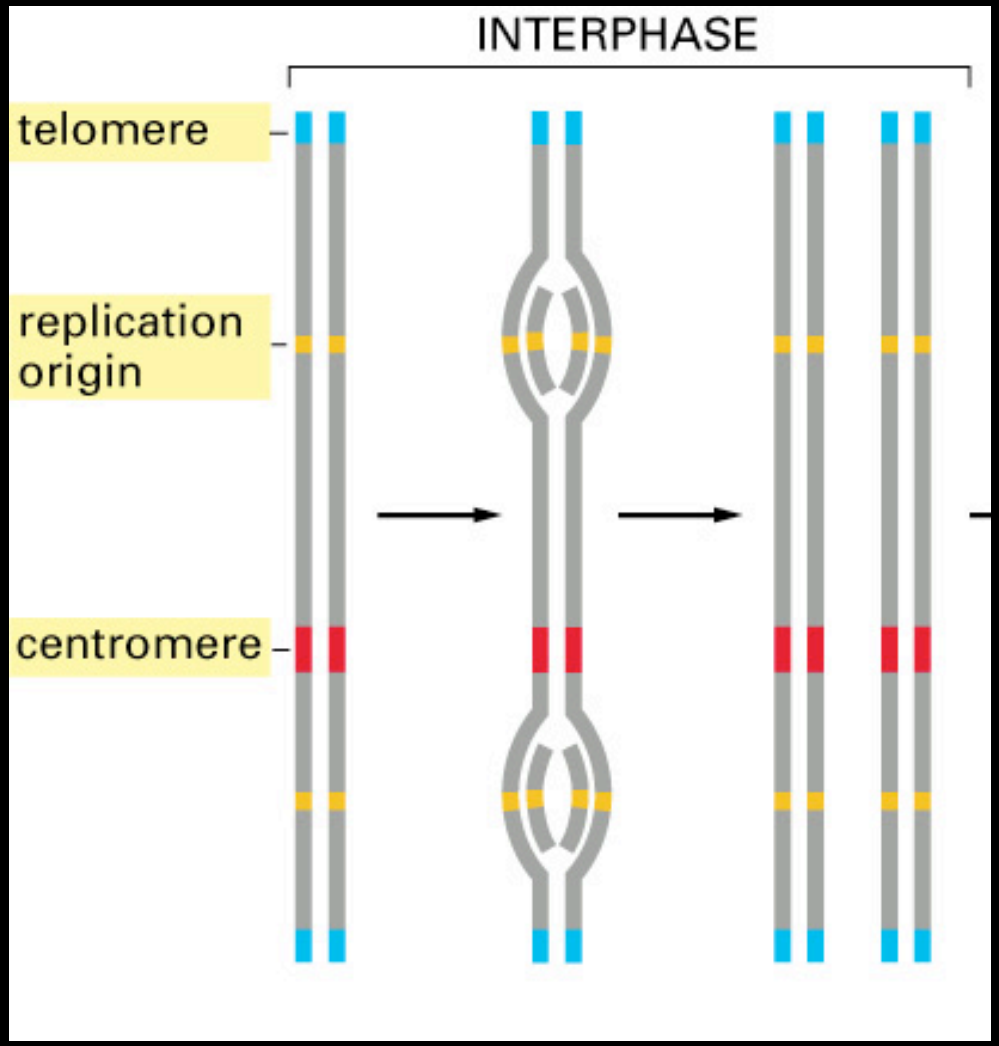
Su(var)3-9 : H3 K9 methyltransferase

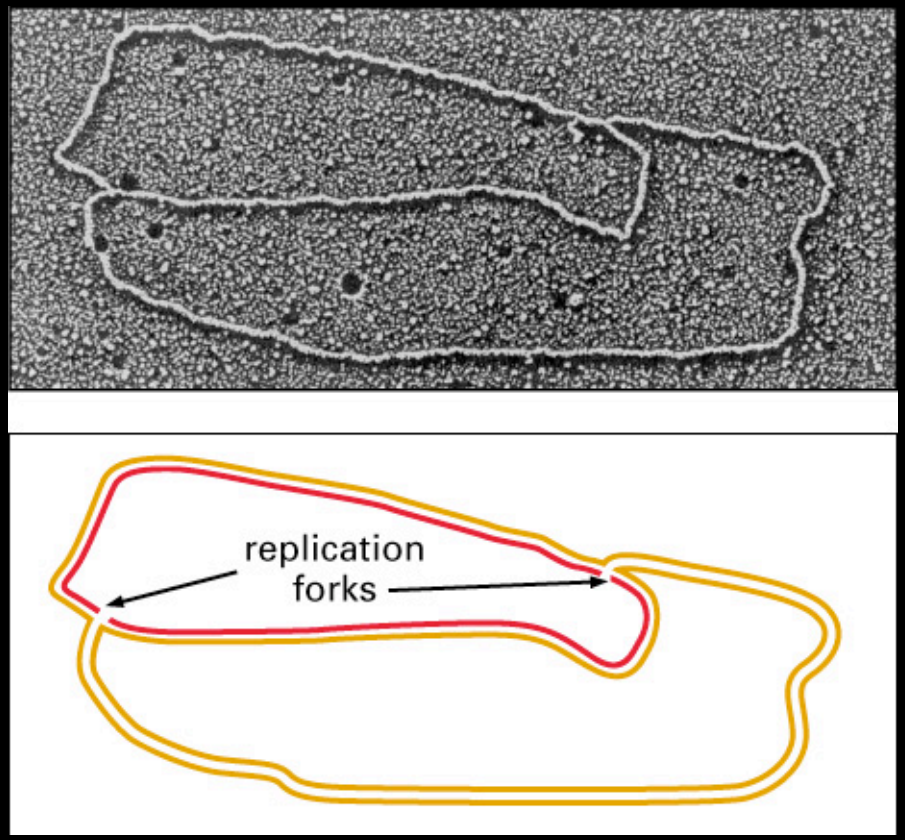
HP1 / Su(var)2-5 : chromodomain, binds H3 K9 Me & 3-9



Jenuwein

Replication is Initiated at ‘Origins’

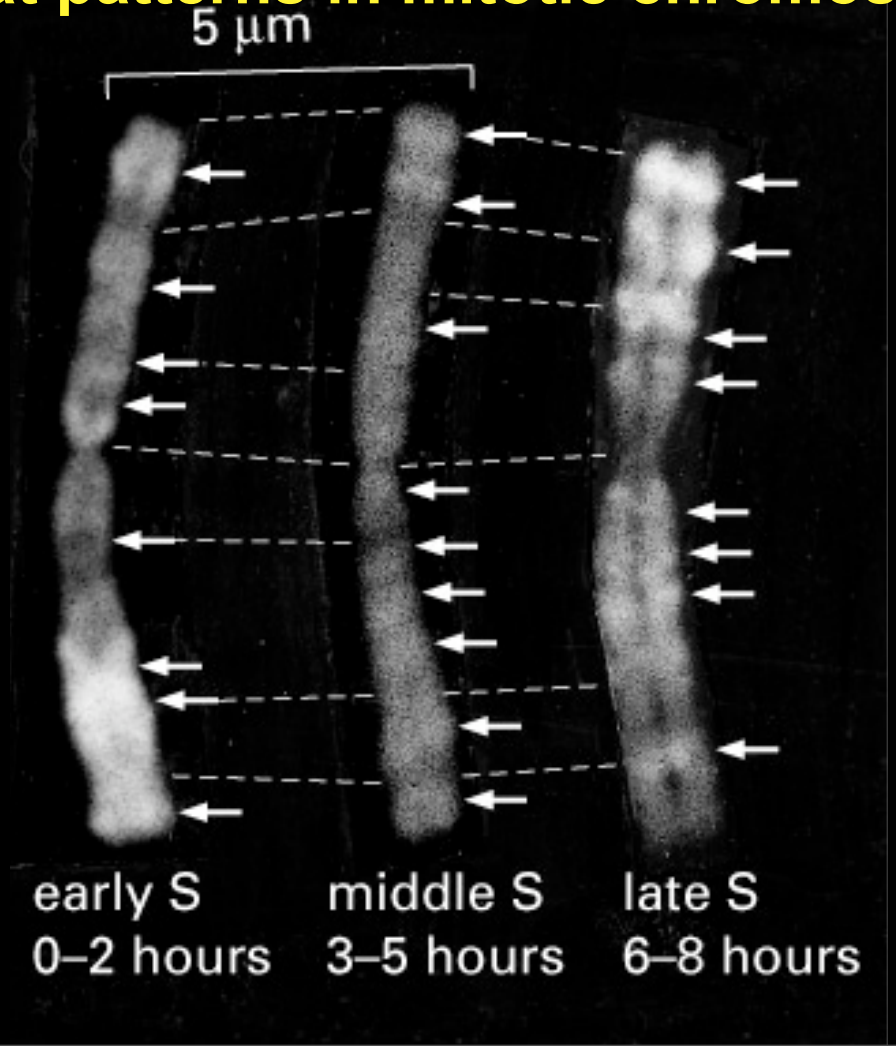




normally sequence dependent in cerevisiae, probably epigenetic in higher euks

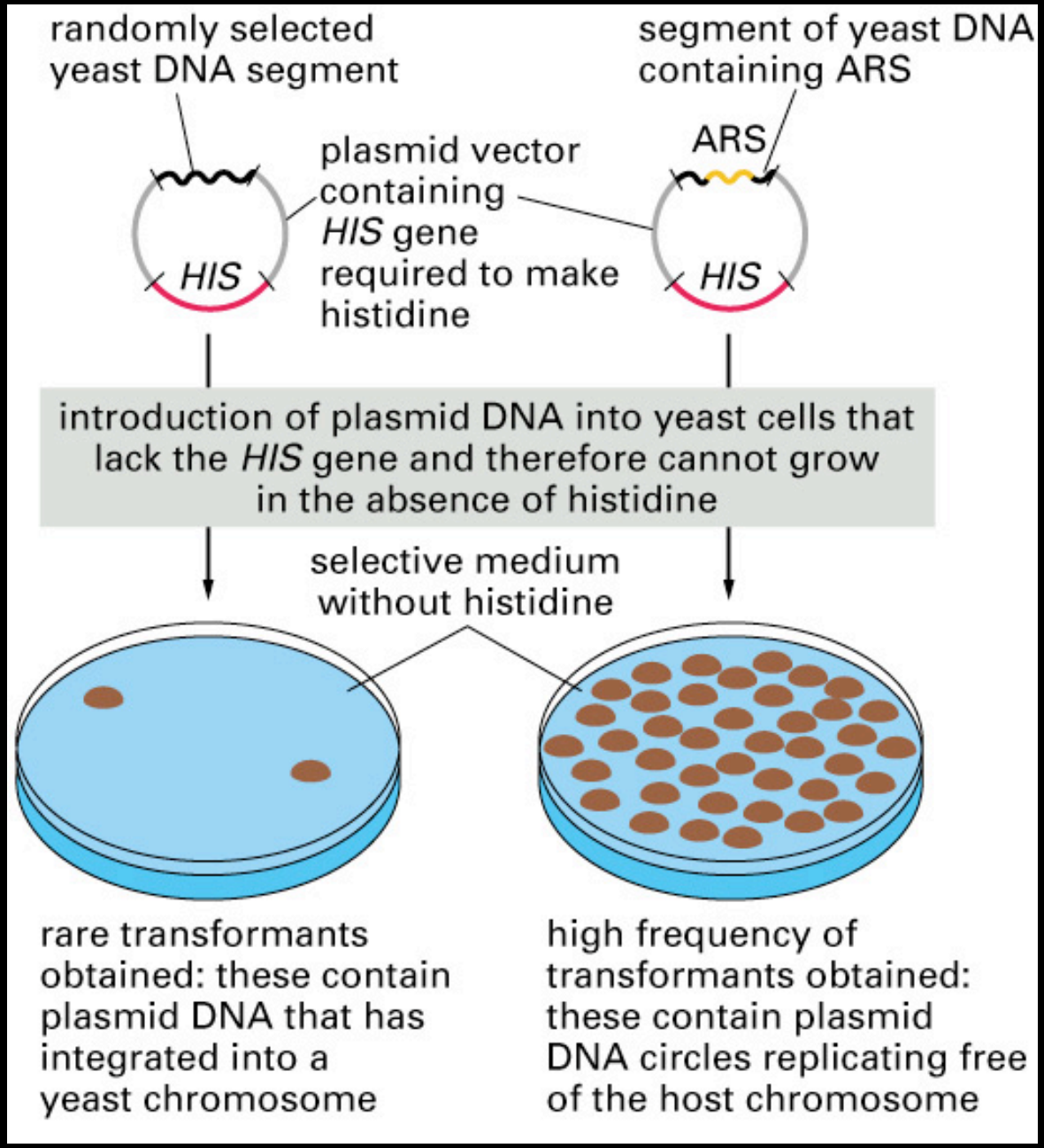
Temporal Control of Replication during S phase

synchronize cells
add labeled nucleotides (e.g. BrdU) at different times in S
look at patterns in mitotic chromosomes



Timing of Replication in Yeast is Chromatin-Dependent

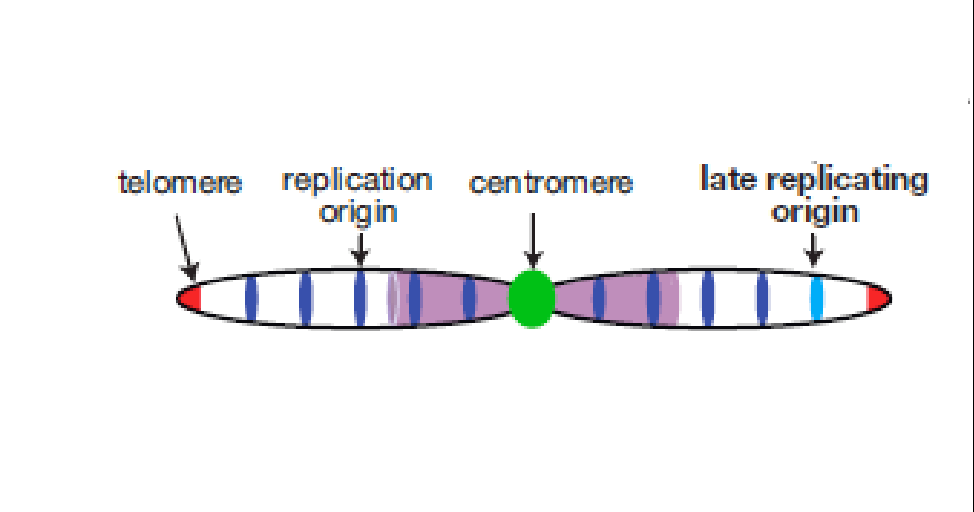
identification of ARSs (Autonomously Replicating Sequences)



Replication Timing and Activity is Chromatin-Dependent in Yeast

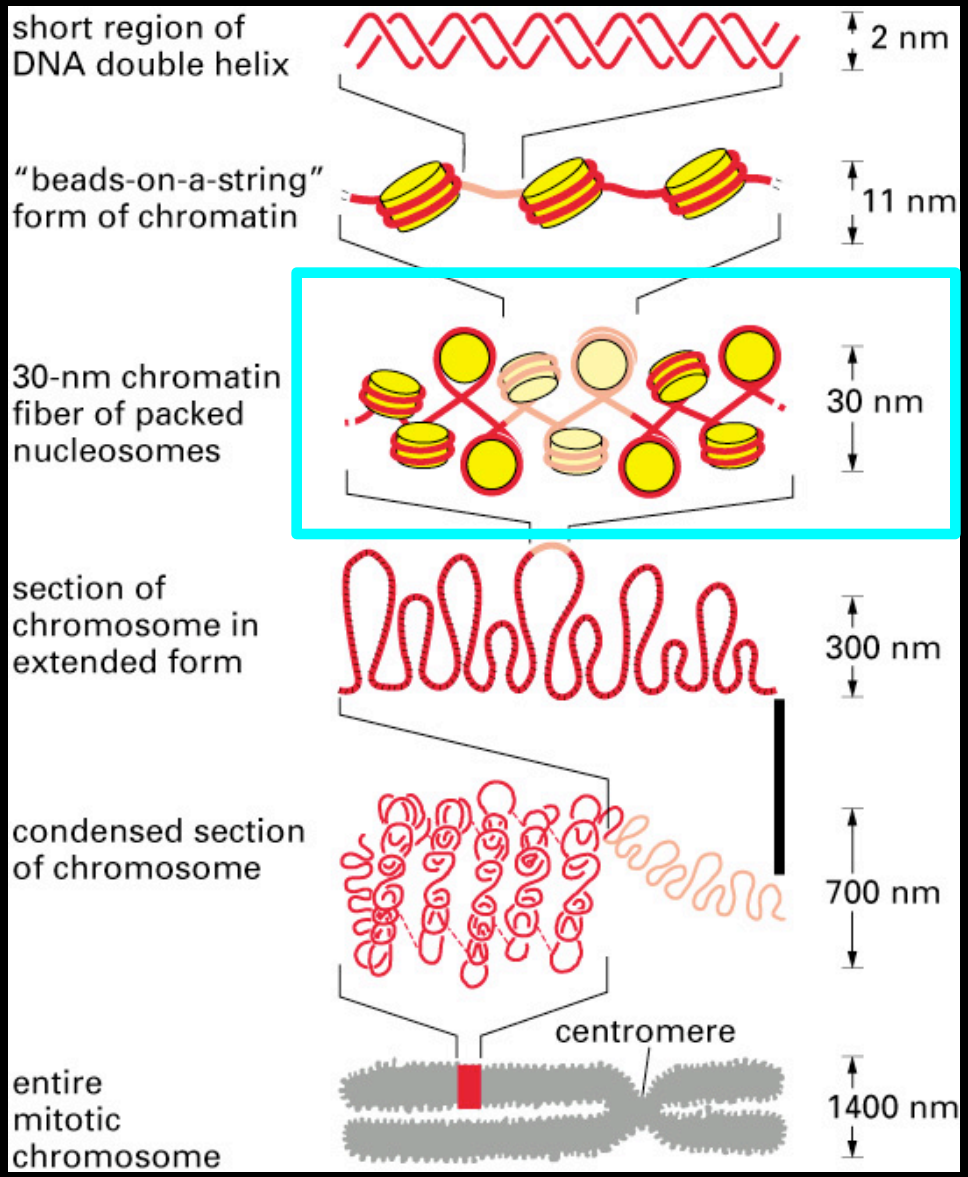
each rDNA copy (in tandem arrays) contain an ARS, but only 20% ‘fire’

telomeric regions ‘silenced’ for gene expression-also replicate late in S



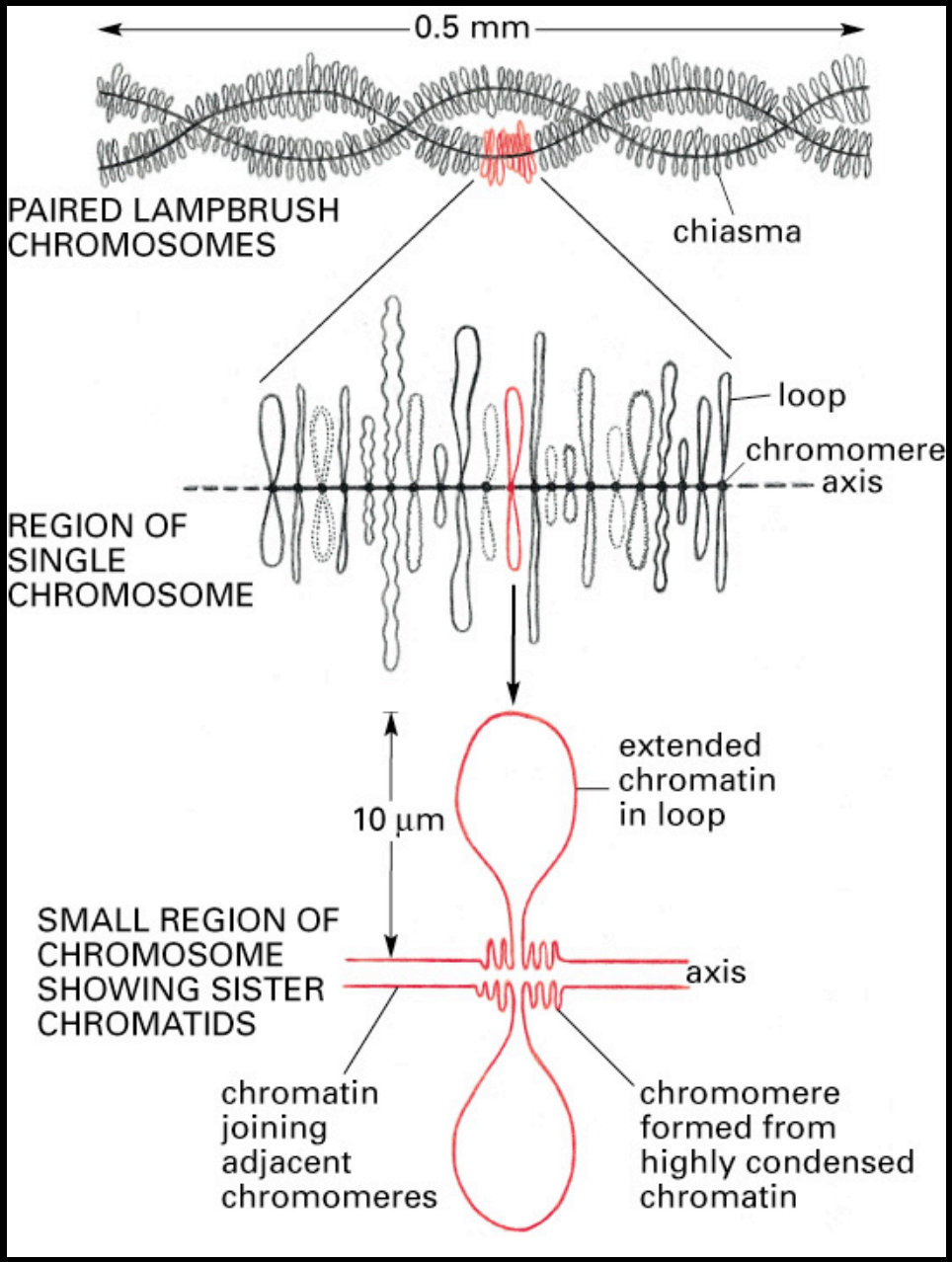
(Ferguson and Fangman 1992; Weinreich et al. 2004)

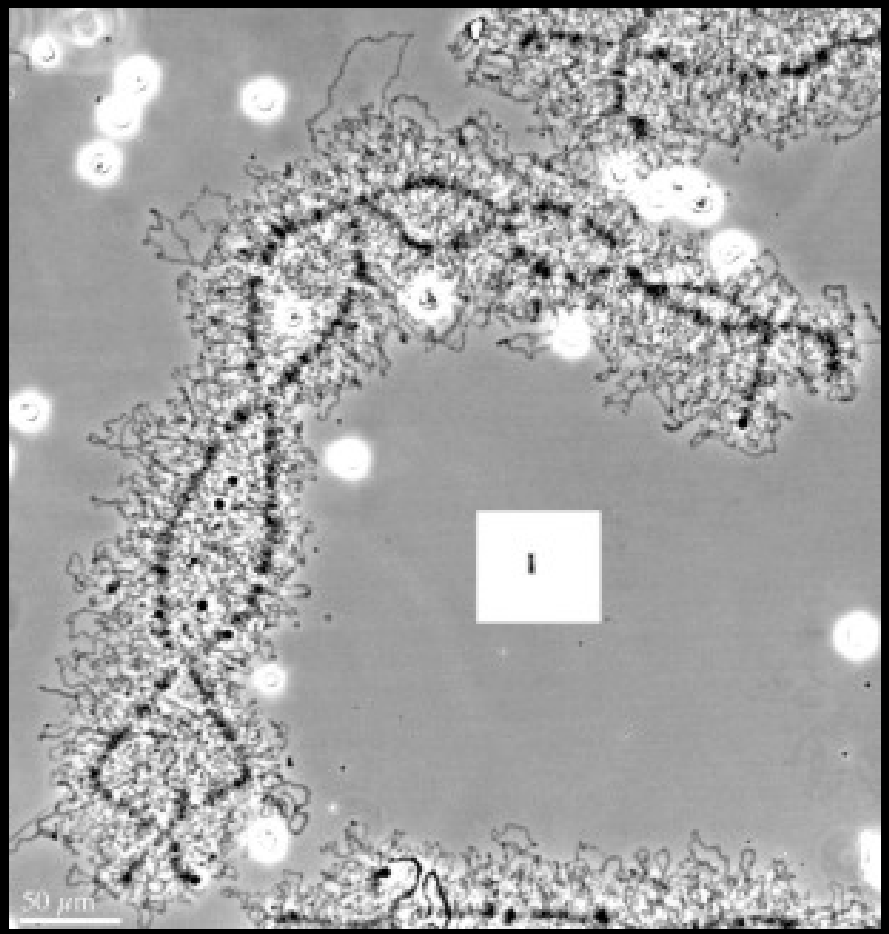
Gene expression is also dependent on chromosome structure and nuclear organization

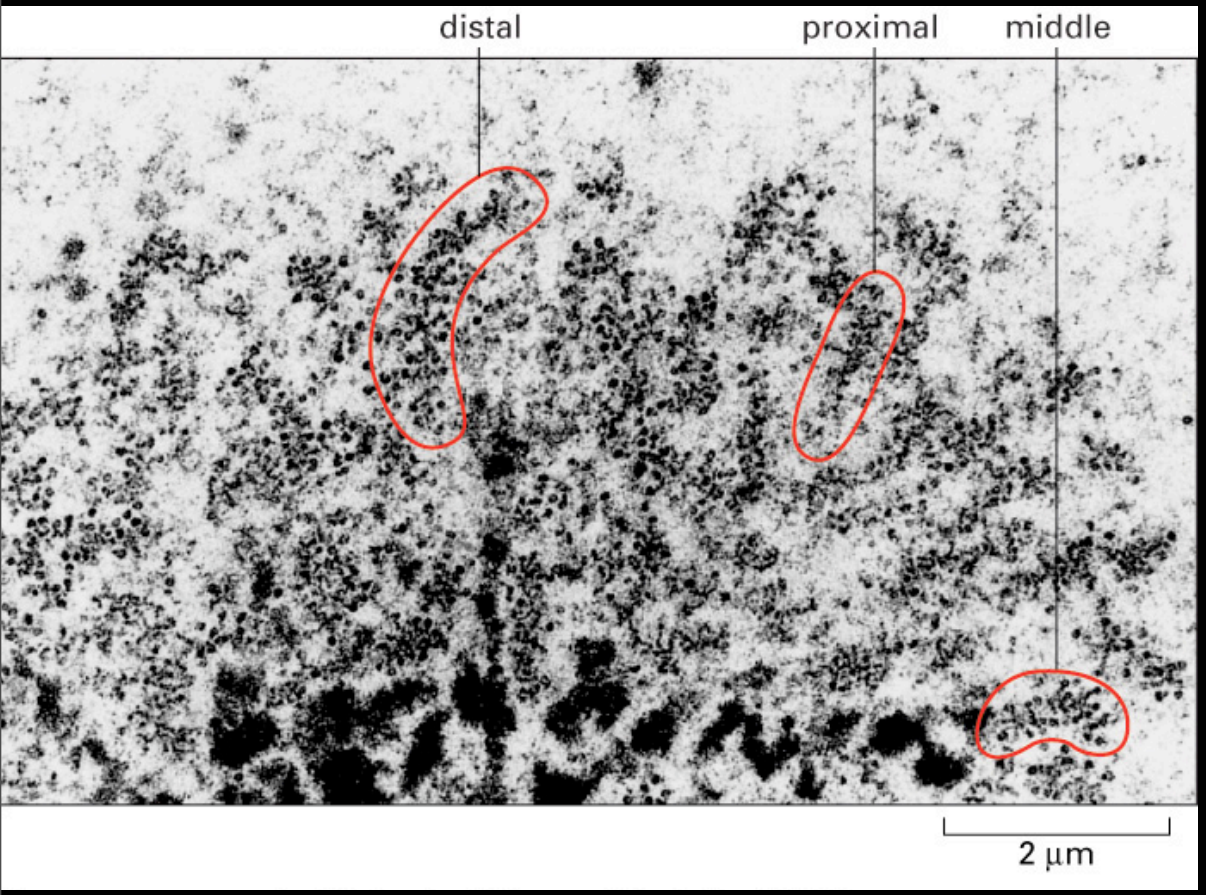


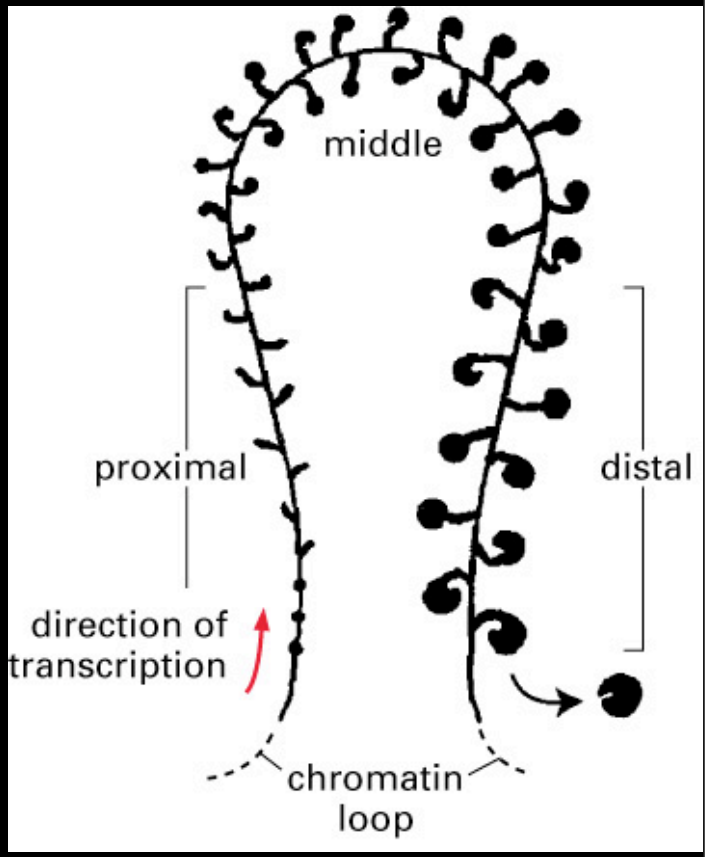
not able to be expressed

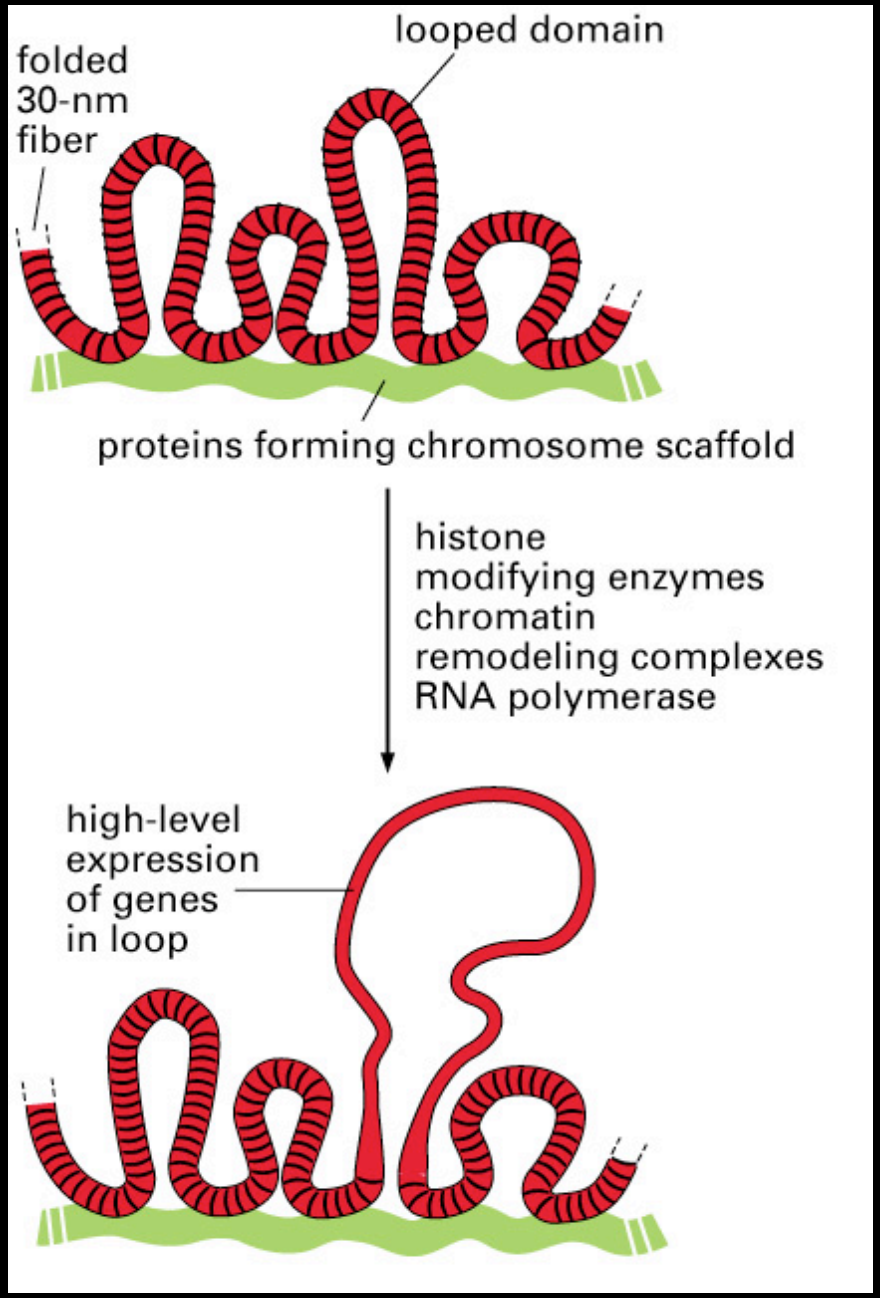
Lampbrush loops in Amphibian Oocytes



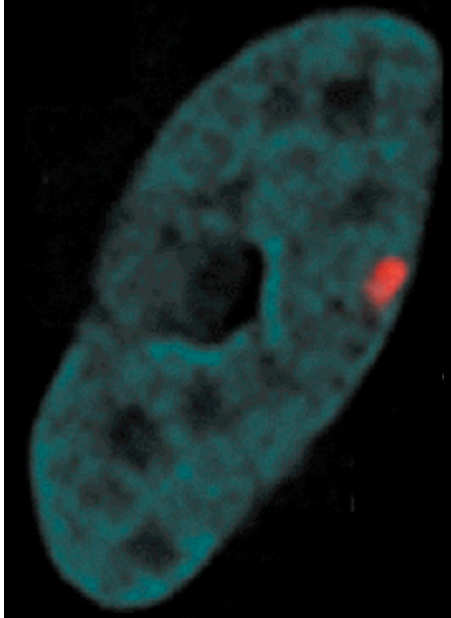



distal
proximal
middle
2 µm


middle
proximal
distal
direction of transcription
chromatin loop



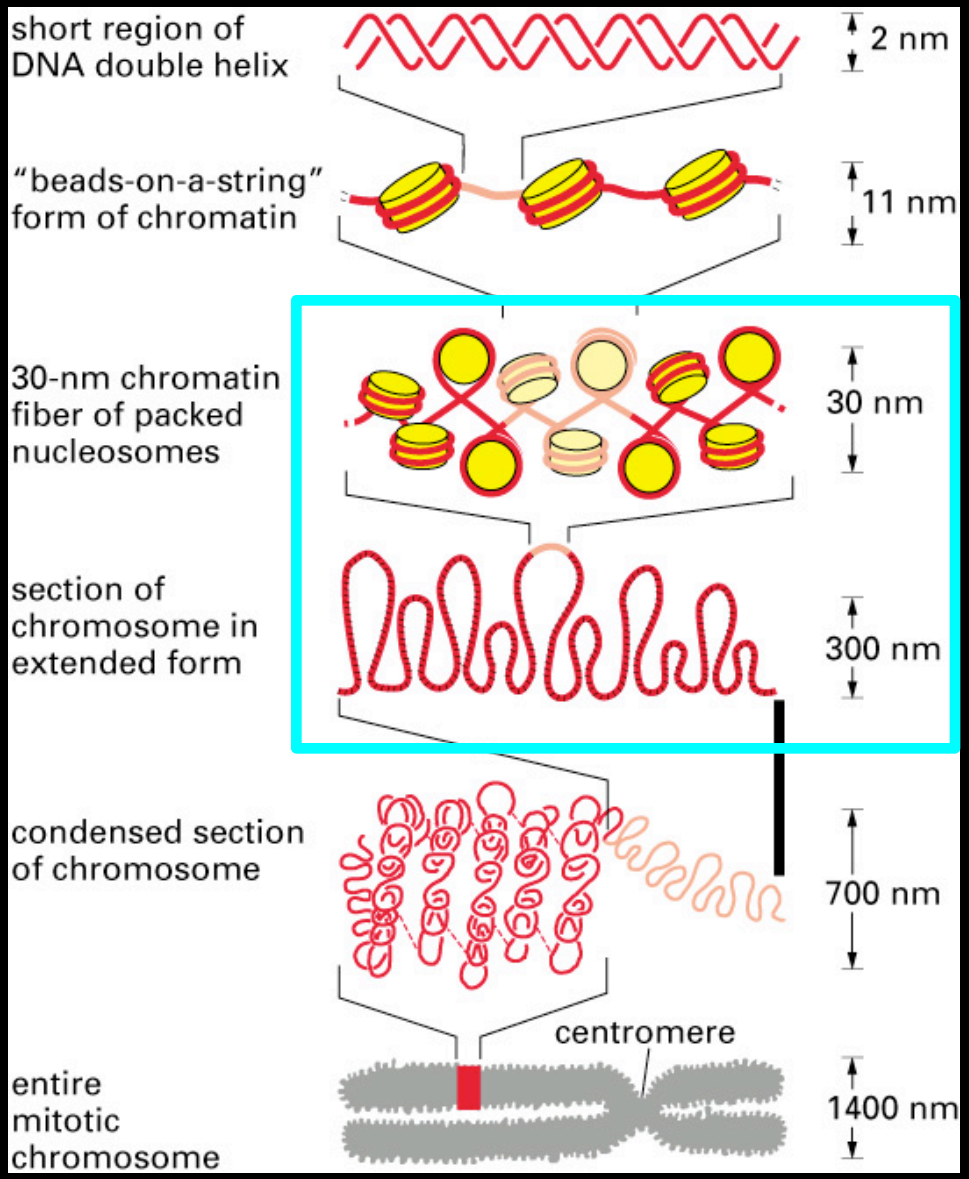
Andy Belmont

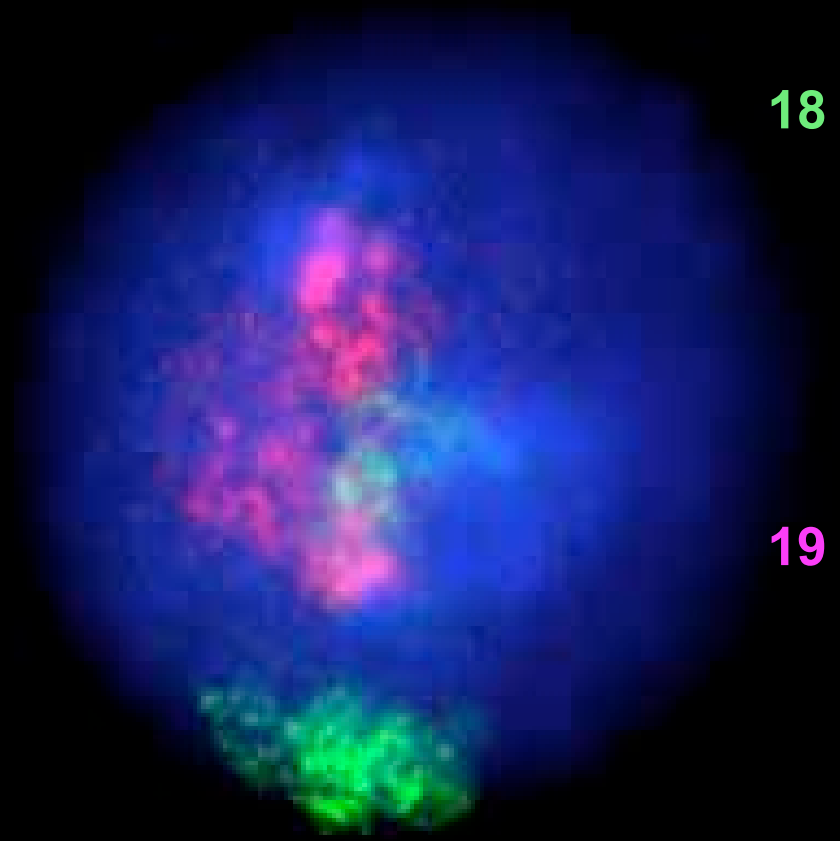


chromosomes marked with lacO sites bound by lacR-RFP

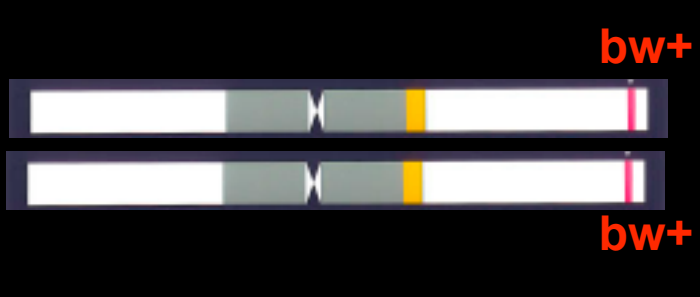
unlooping observed in real time after tx activation

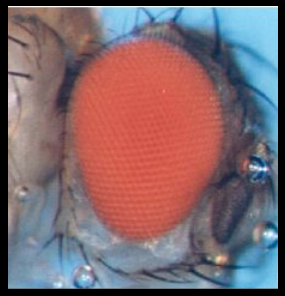
Effects on expression in the context of nuclear architecture





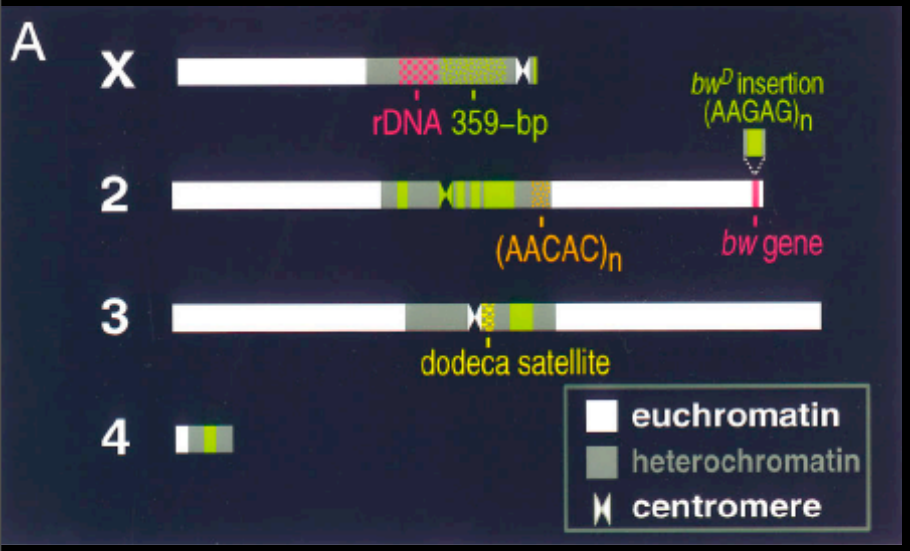
Brown-Dominant: A model for effects of nuclear organization of chromosomes on gene expression

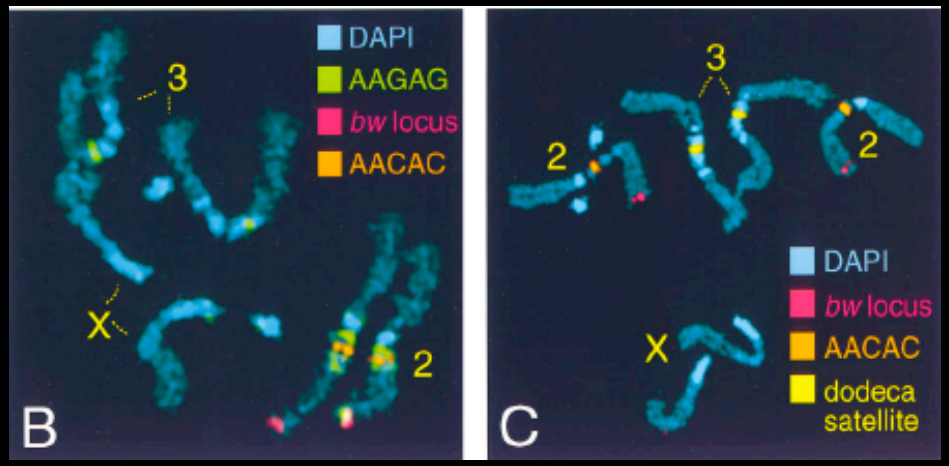




bw expressed

Dernburg

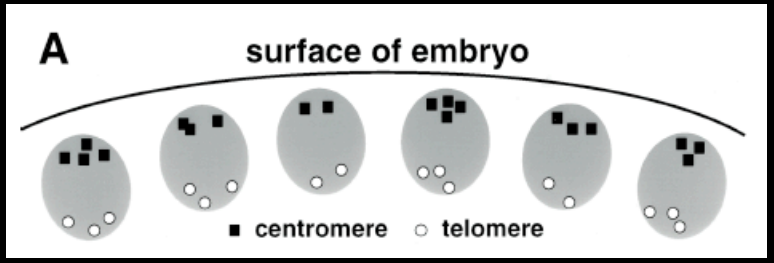


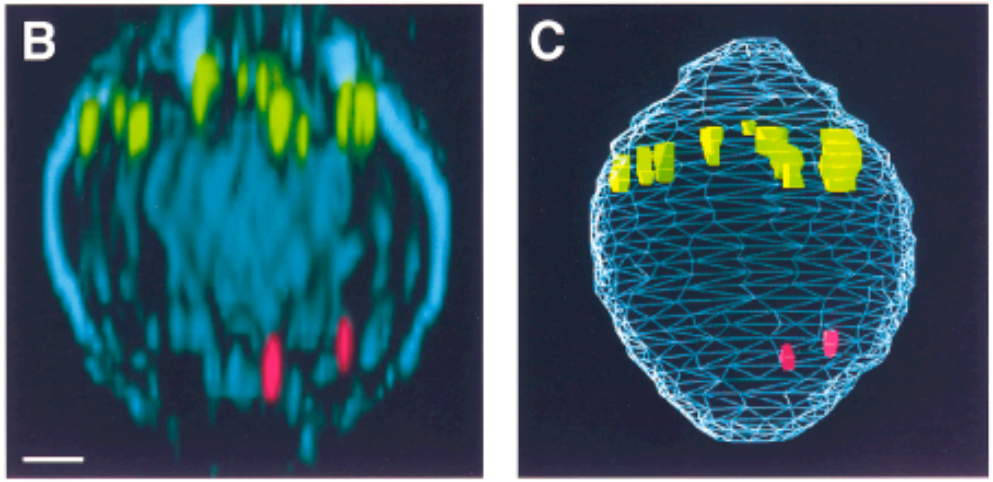


use FISH probes-mark positions of bw and heterochromatin (satellites)

bw

AAGAG



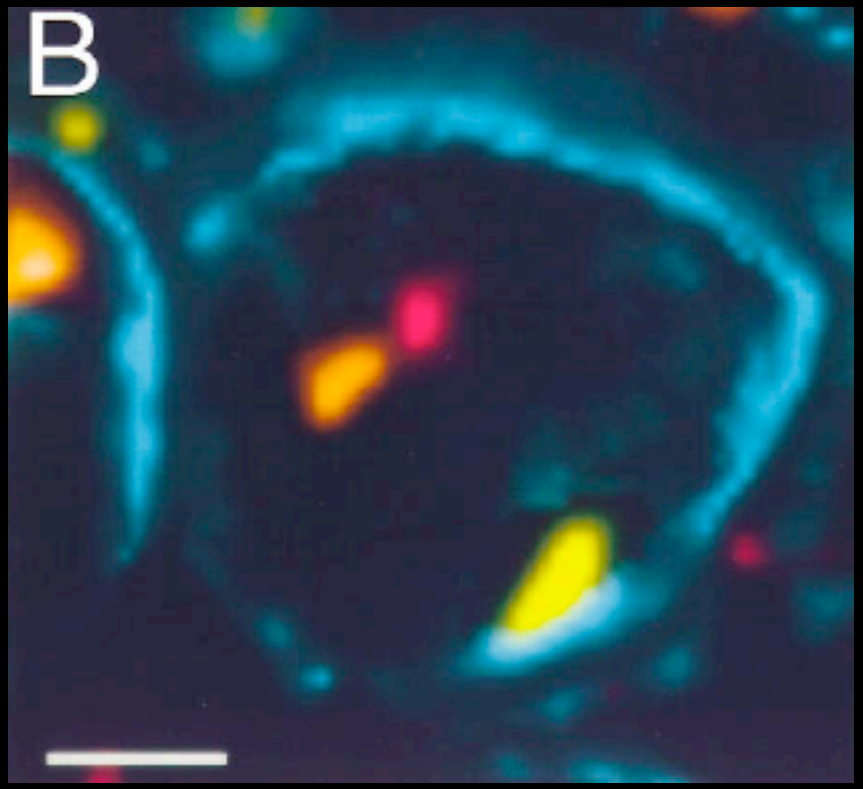


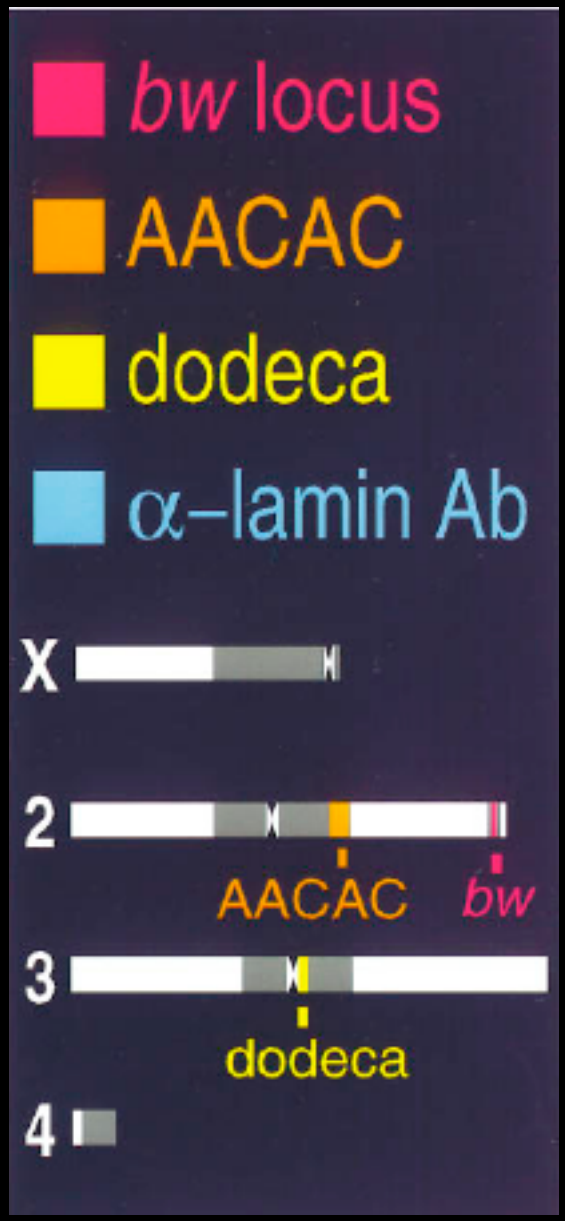
nucleus from a wild–type embryo

bw normally basal,
AAGAG apical

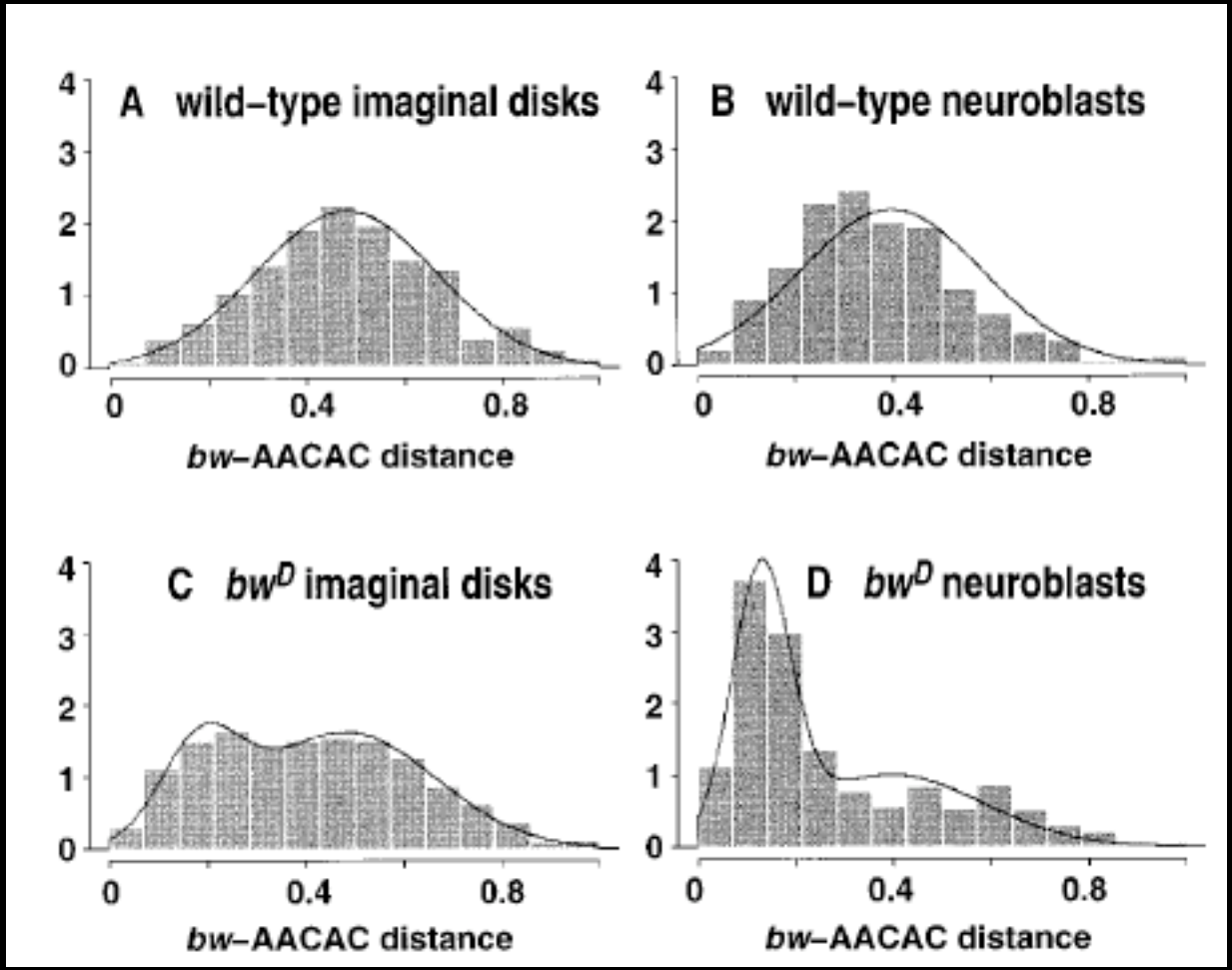
Brown+ associates with heterochromatin in tissues relevant to eye expression

larval imaginal discs (eye precursor)





association is specific to the SAME chromosome, not heterochromatin in general

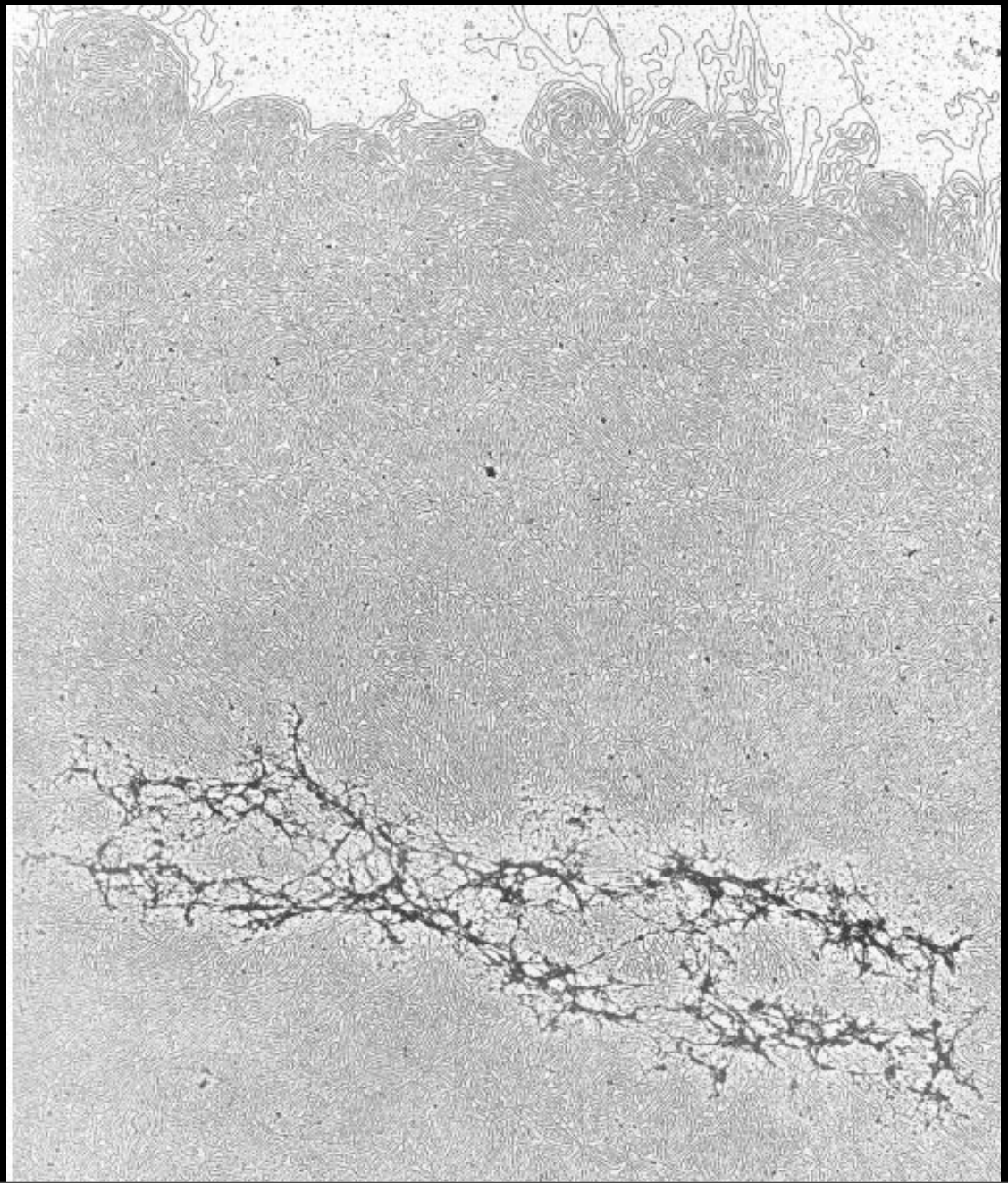


bw+ normally in euchromatic ‘compartment’
bw-D ‘loops’ in cis to associate with 2 heterochromatin, due to AAGAG insertion

bw+ / bw-D - bwD associates in trans with bw+, ‘loops’ bw+ in trans to associate with 2 heterochromatin and silence gene expression of bw+

Mitotic chromosome structure and function

High Salt extraction of chromosomes reveals ‘scaffold’



Condensins and Cohesins

~1990- cerevisiae screens for defective chromosome inheritance

genes required for
Structural Maintenance of Chromosomes (SMC proteins)

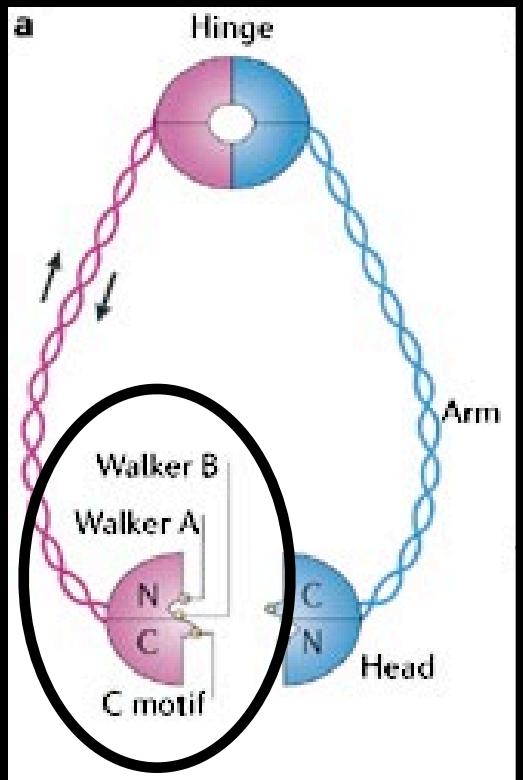
conserved from bacterial to human, ATPases

crucial roles in chromosome segregation (mitosis and meiosis)

chromosome-wide gene regulation and recombinational repair

novel type of protein machine : function as dynamic linkers of the genome

large- 1000-1300 aa
monomers fold back on themselves (antiparallel coiled-coil)
exist as dimers

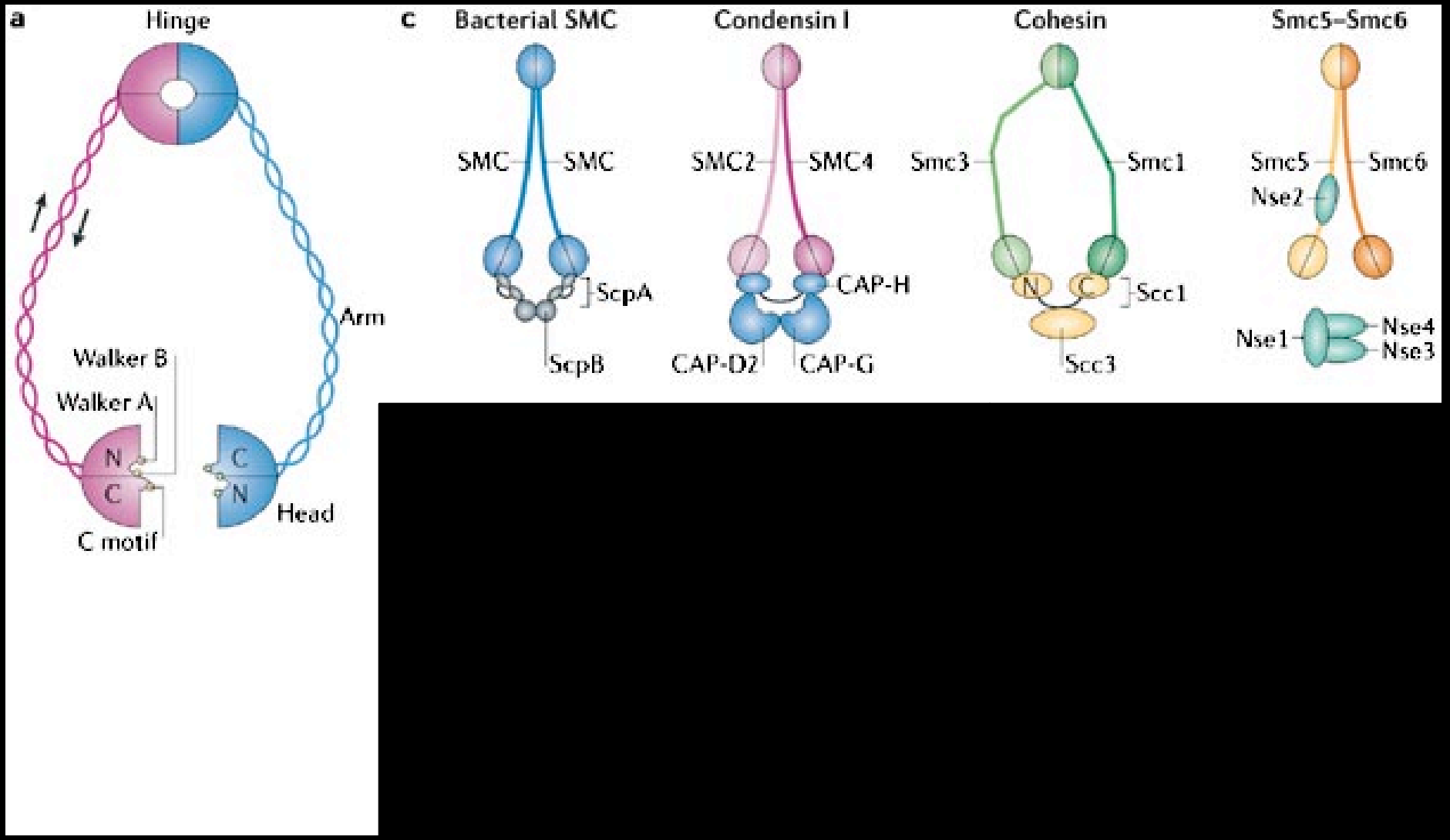


nucleotide binding sites at N and C termini

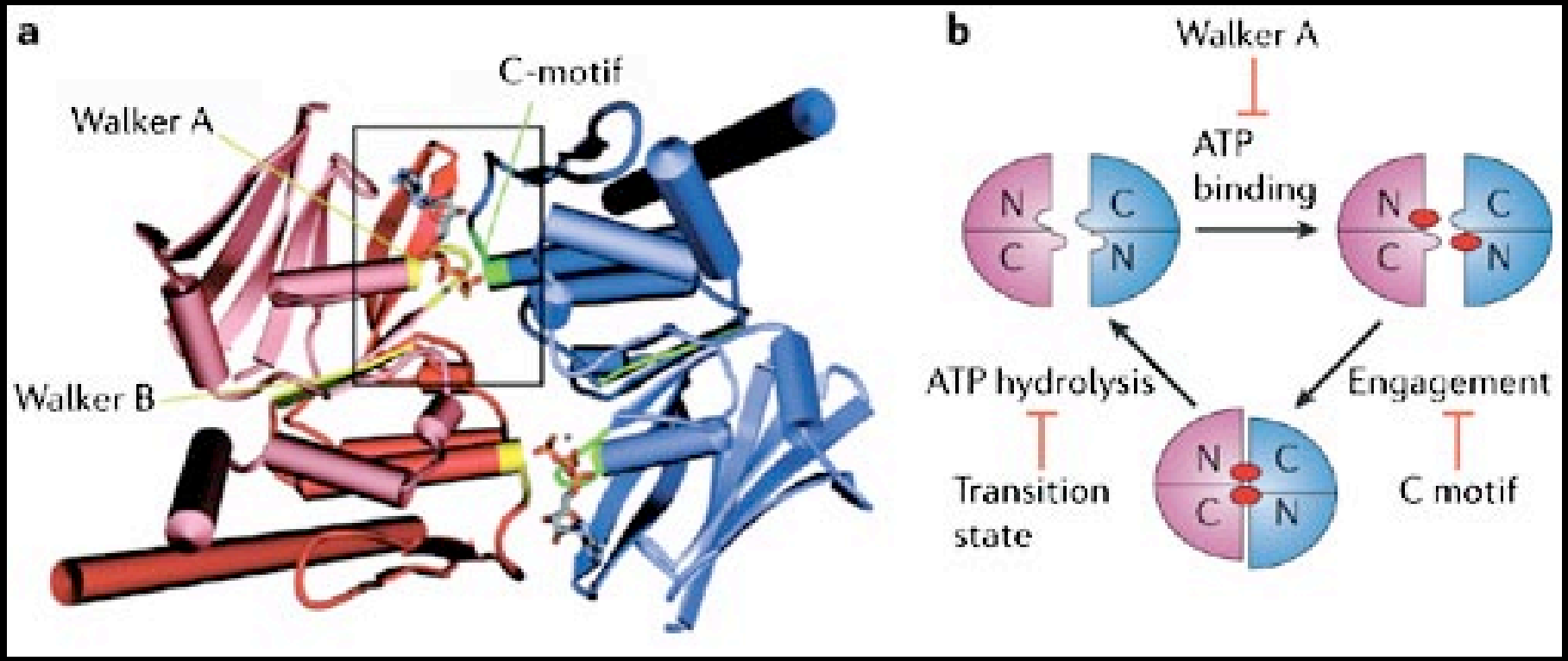
different SMCs in complexes associated with different functions
each contains non-SMC proteins, associated with heads

regulate catalytic activity and interactions

repairin?

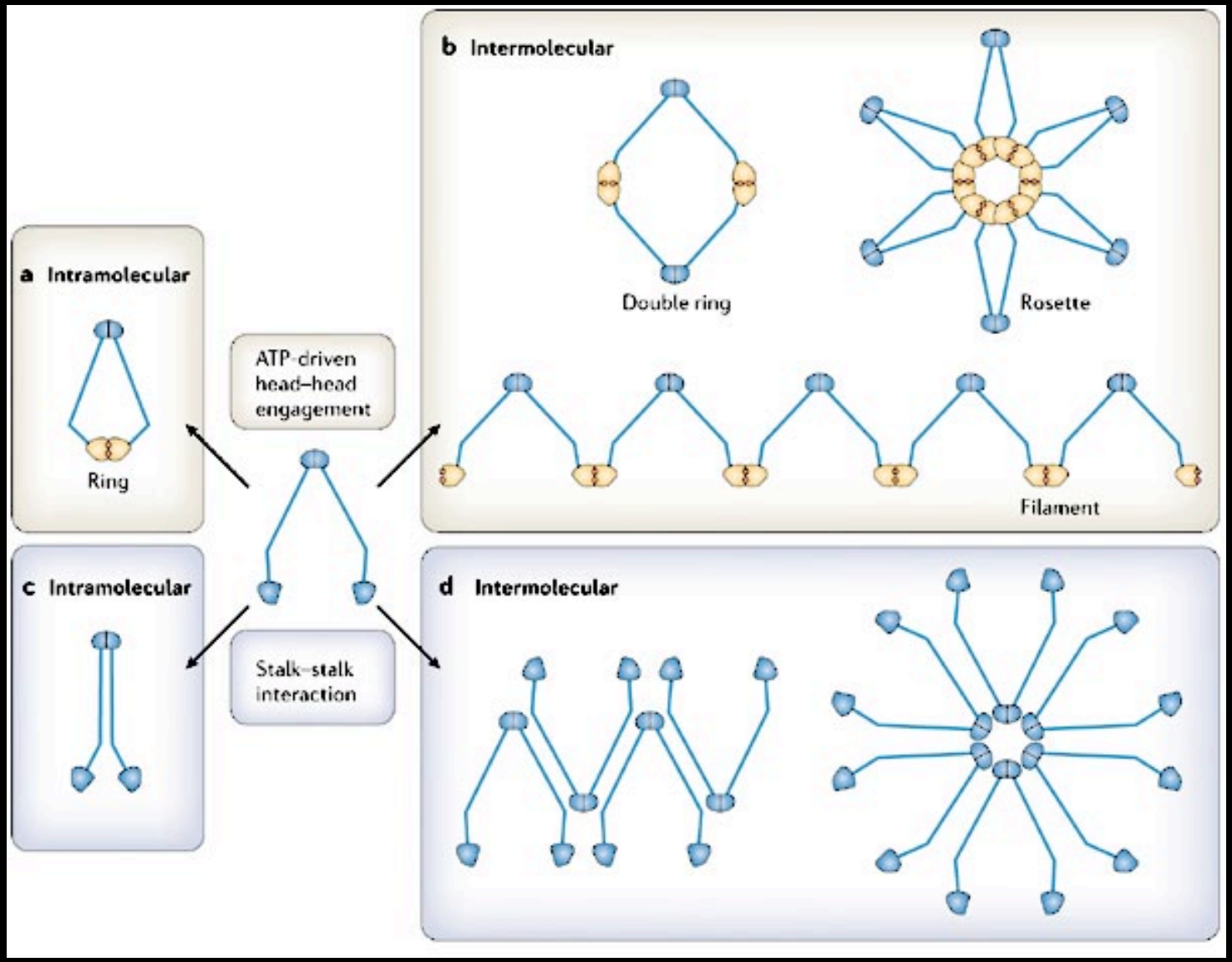


ATP binding drives head associations
associations required for ATP hydrolysis
ATP hydrolysis required for disengaging heads



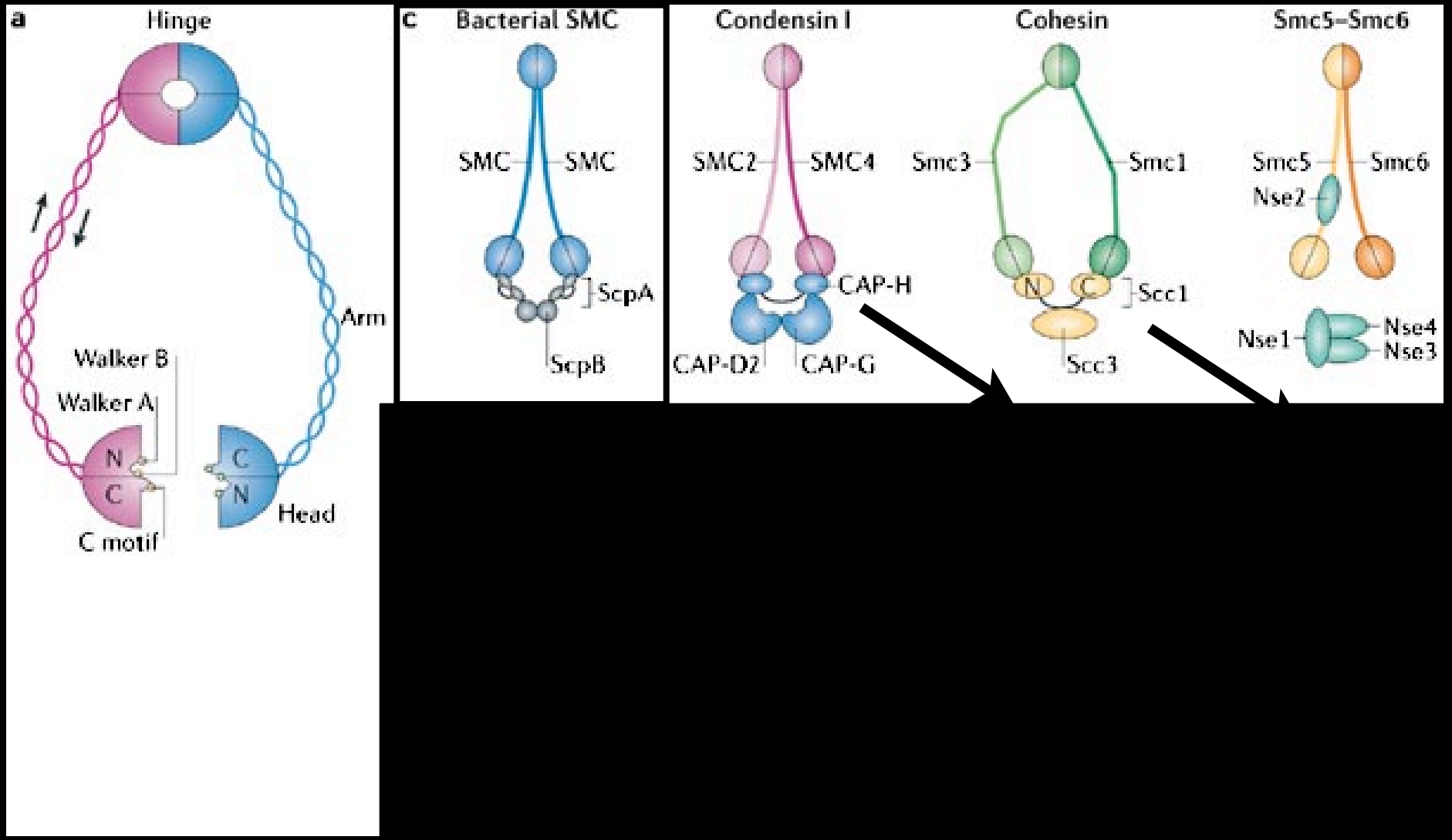
hinge associations strong, independent of ATP

many types of possible intra- and inter- molecular interactions can form different higher order complexes and structures



intermolecular interactions require DNA?

Structures observed in vitro by EM



different for condensin and cohesin

Condensin

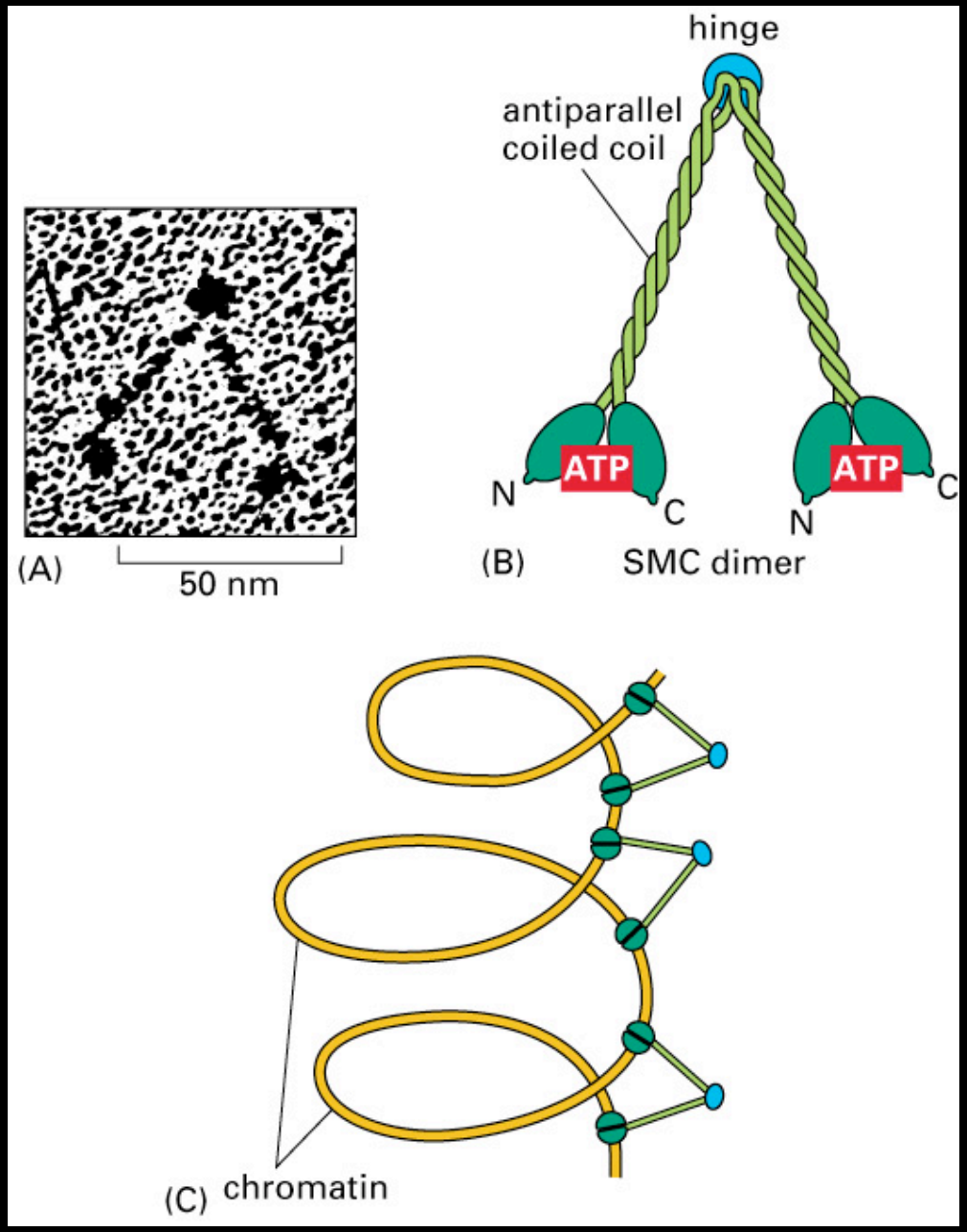
two complexes:
Condensin I and II

both have SMC 2 and 4
different non-SMC components

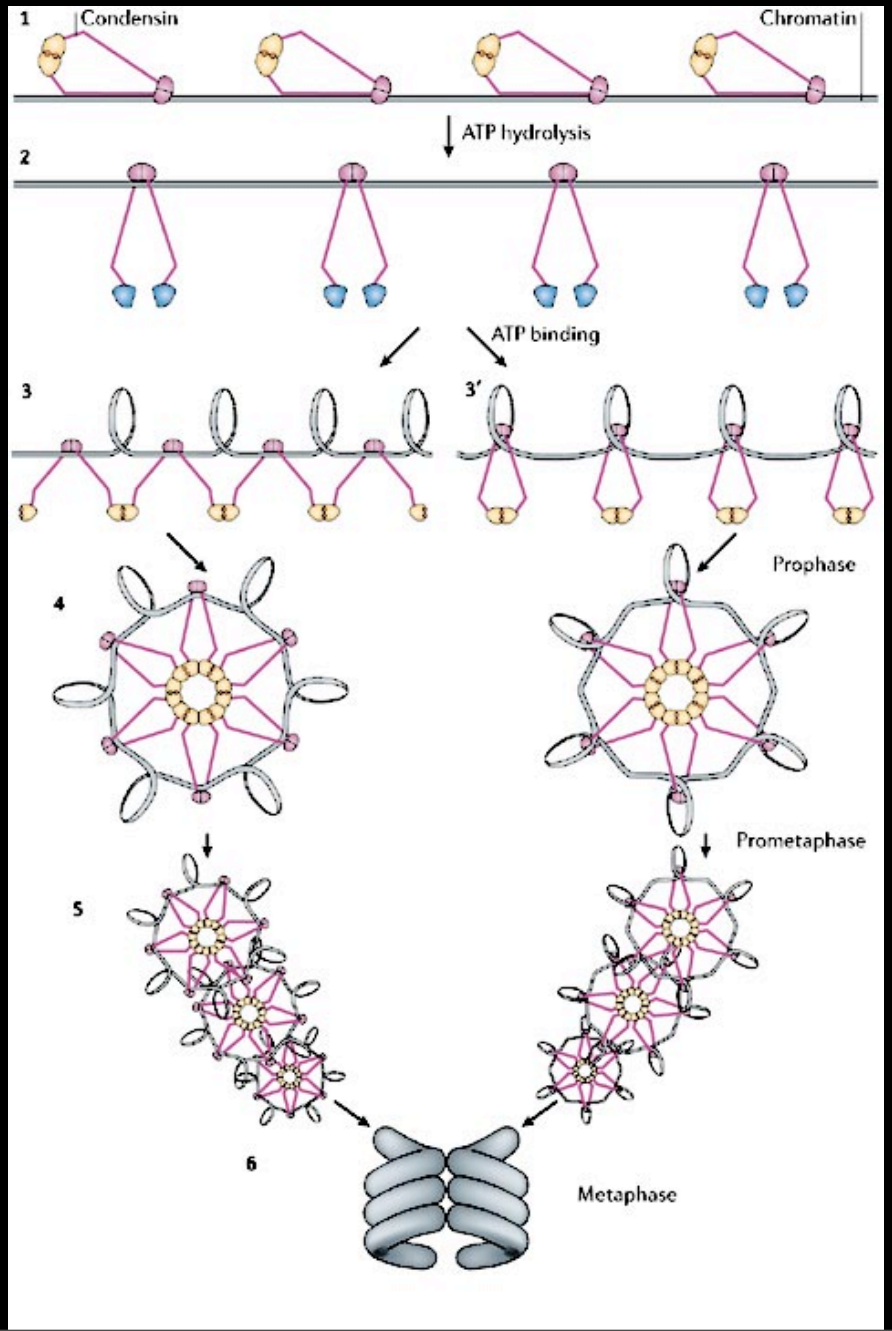
bind DNA cooperatively,
not ATP dependent

could also involve the
stalk

Condensin I + ATP drives
positive superhelical tension
on DNA



Speculative model for chromosome condensation by Condensin



acts on chromatin, not DNA

roles of non-SMC subunits?

Cohesin

holds sister chromatids together through metaphase

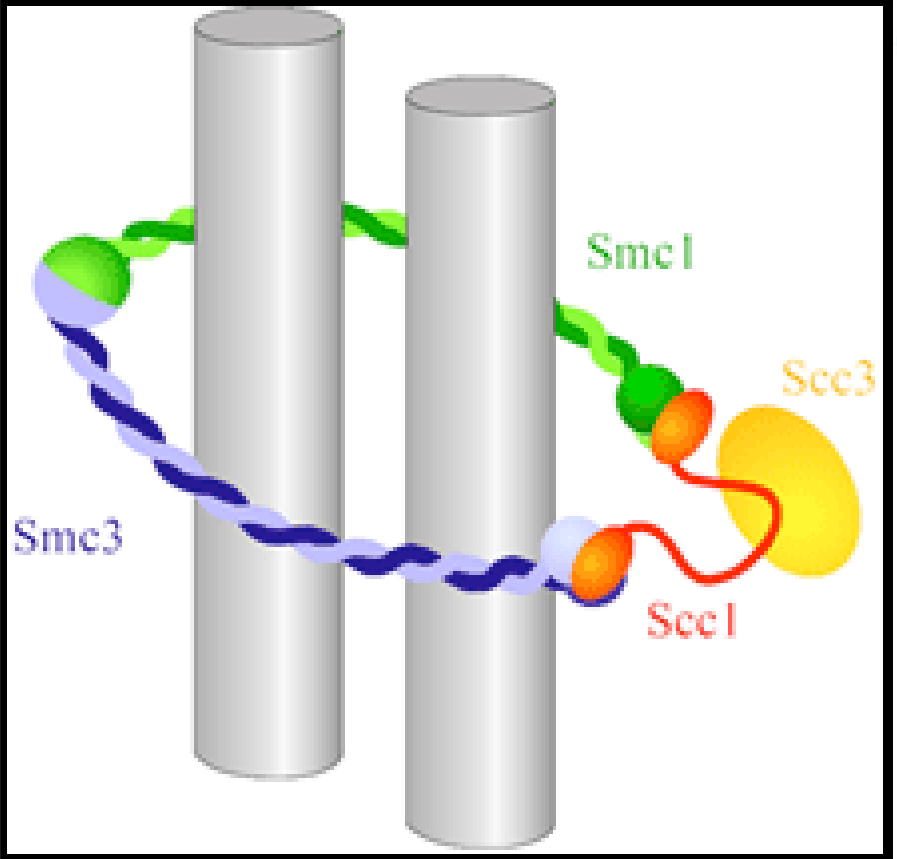
INTERmolecular linking of two DNAs (compare to condensin)

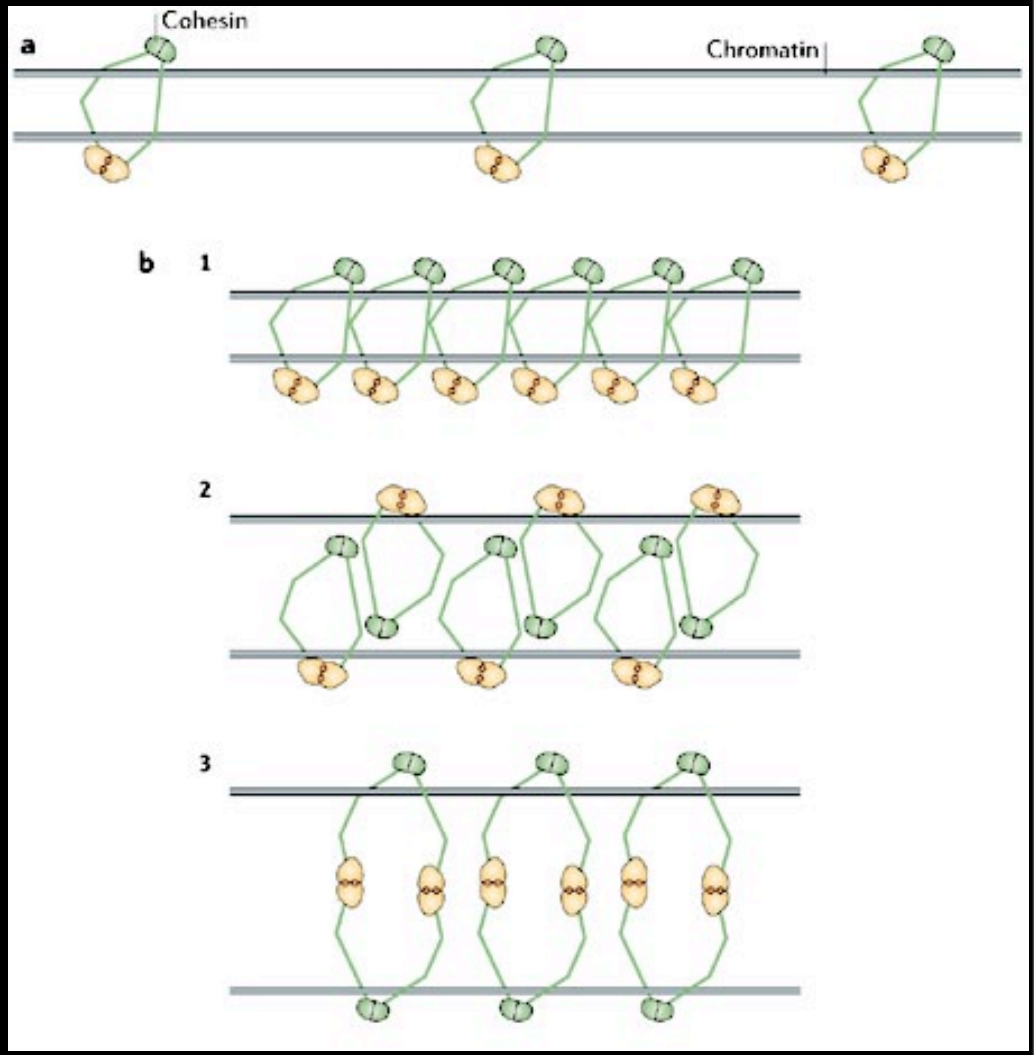
established at replication fork-preloaded in G1?

degraded at onset of anaphase to allow sister separation

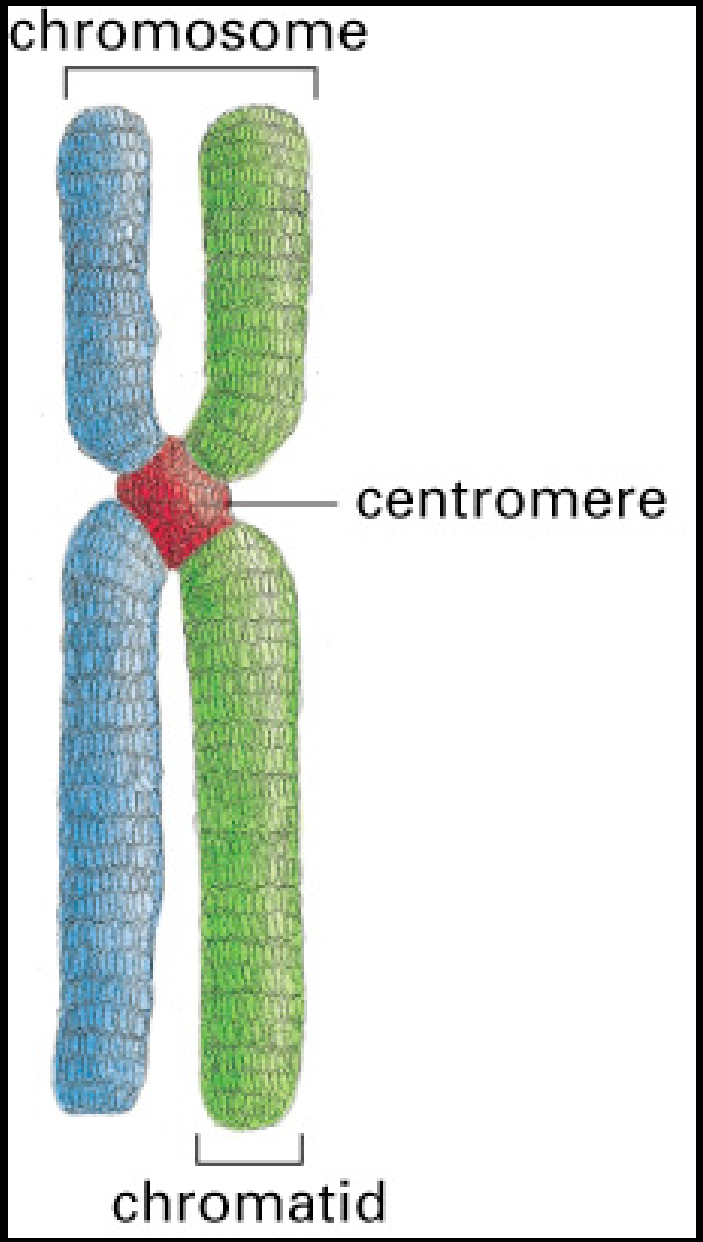
cohesin in pericentromeric regions recruited by HP1/K9me, may be regulated differently

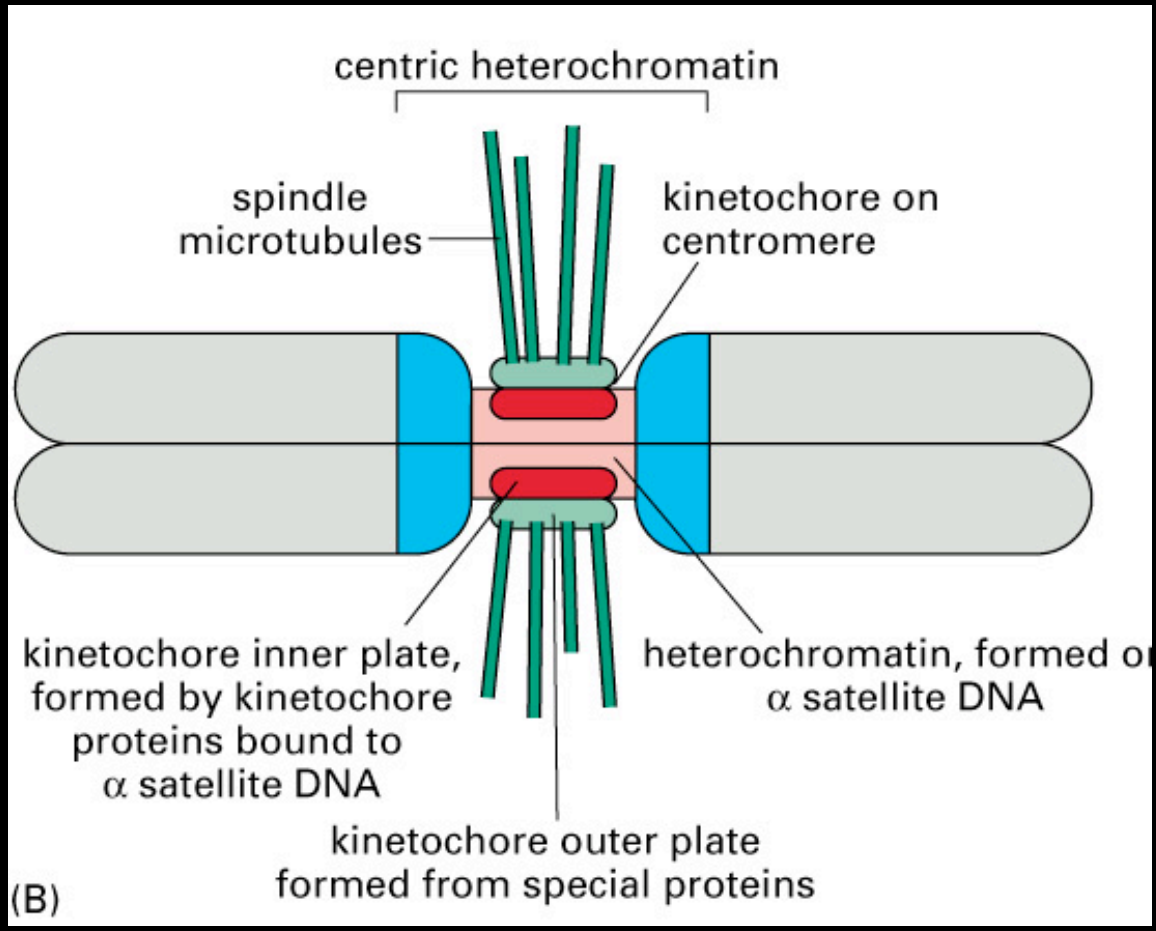
Ring model for Cohesin





Centromeres and Kinetochores





prometaphase and anaphase movement

spindle assembly checkpoint (SAC)

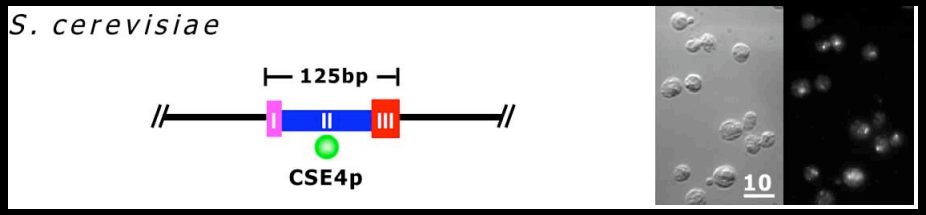
centromere vs. flanking pericentric heterochromatin

there can only be one.....

Centromeric DNA sequences are not conserved

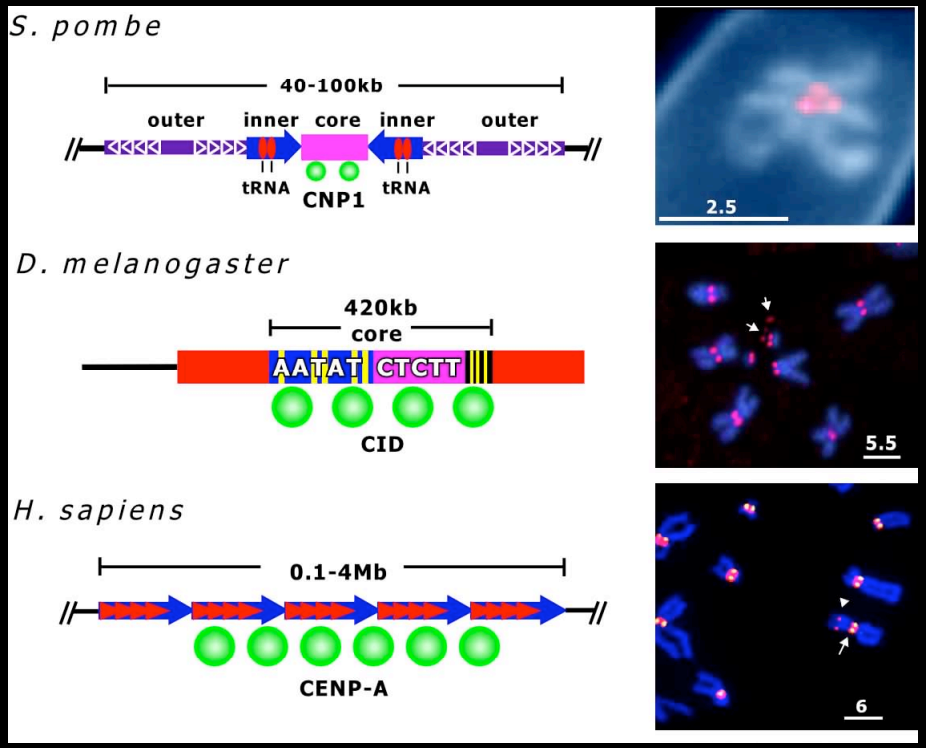
S. cerevisiae and other budding yeast

same specific, 125 bp sequence present at all 16 centromeres
necessary and sufficient for kinetochore formation



everywhere else, often repetitive, 10s-1000s of kb.....but....

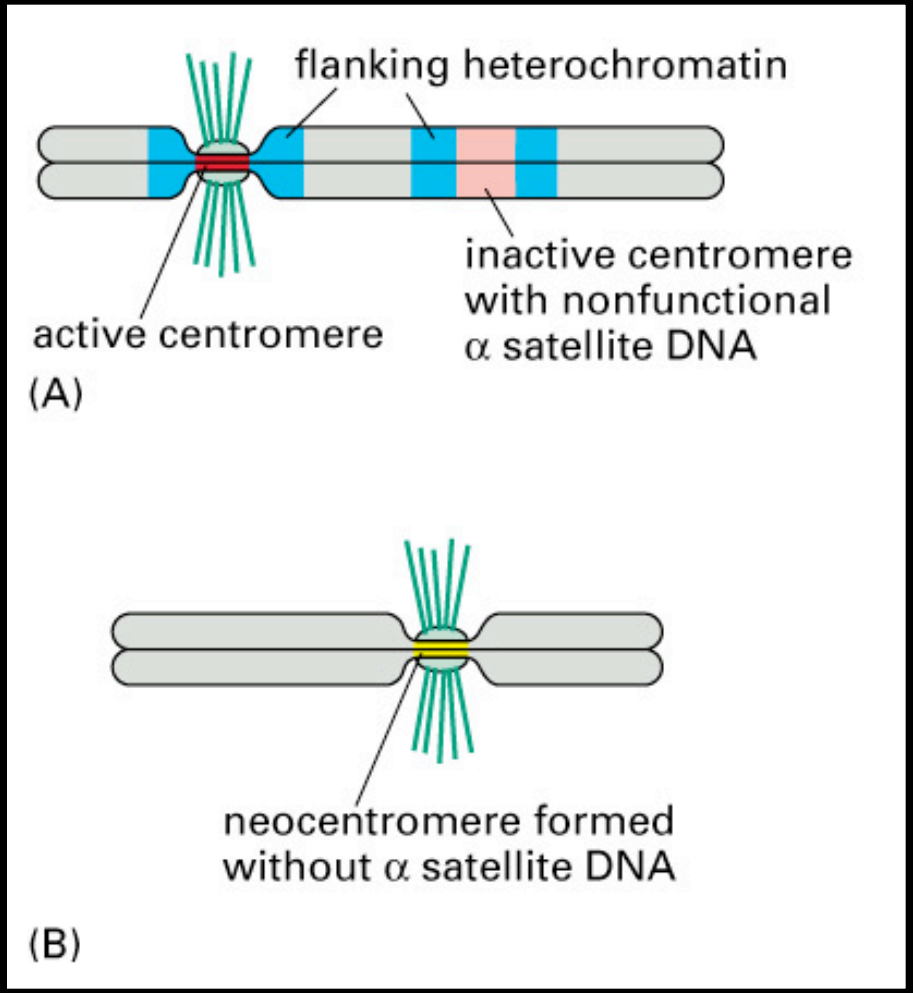
sequences associated with centromeres are not conserved across species, or even among different chromosomes in the same organism

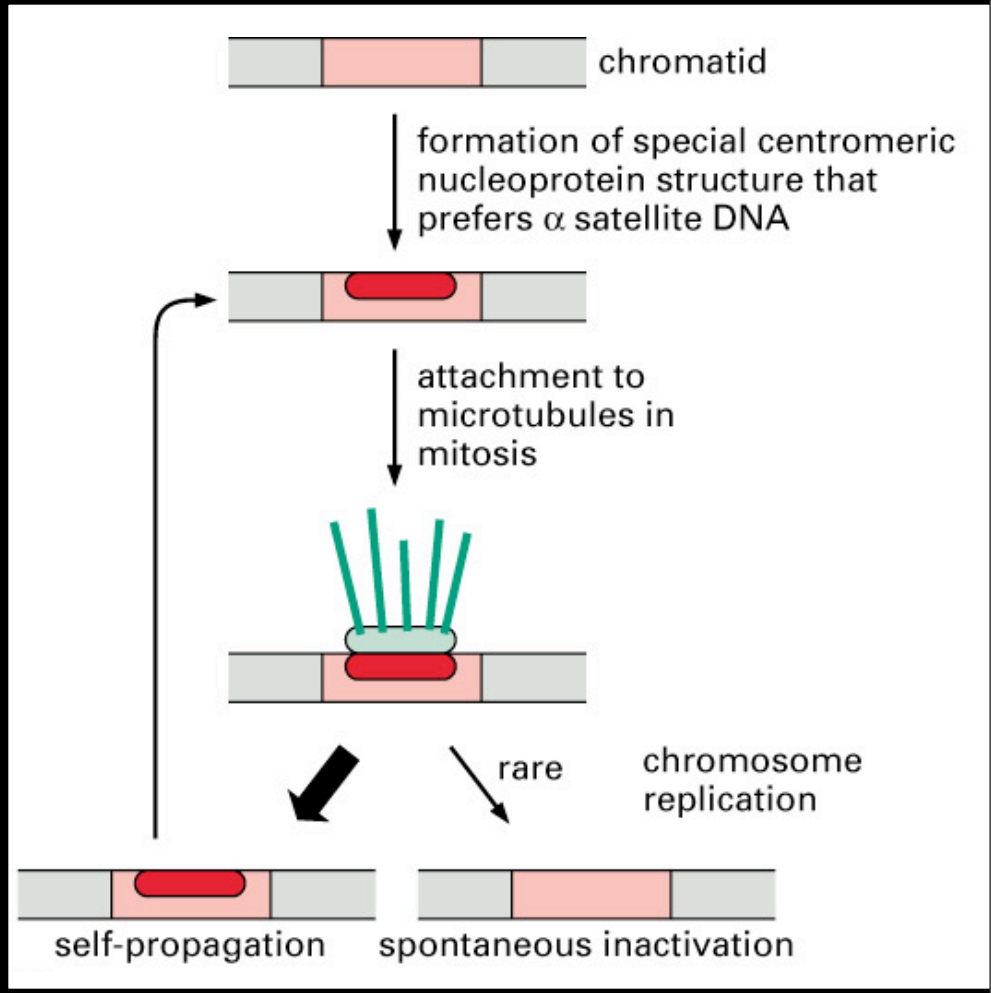


but centromere proteins are highly conserved

Centromere Identity and Propagation are Epigenetically Regulated

Centromere Plasticity

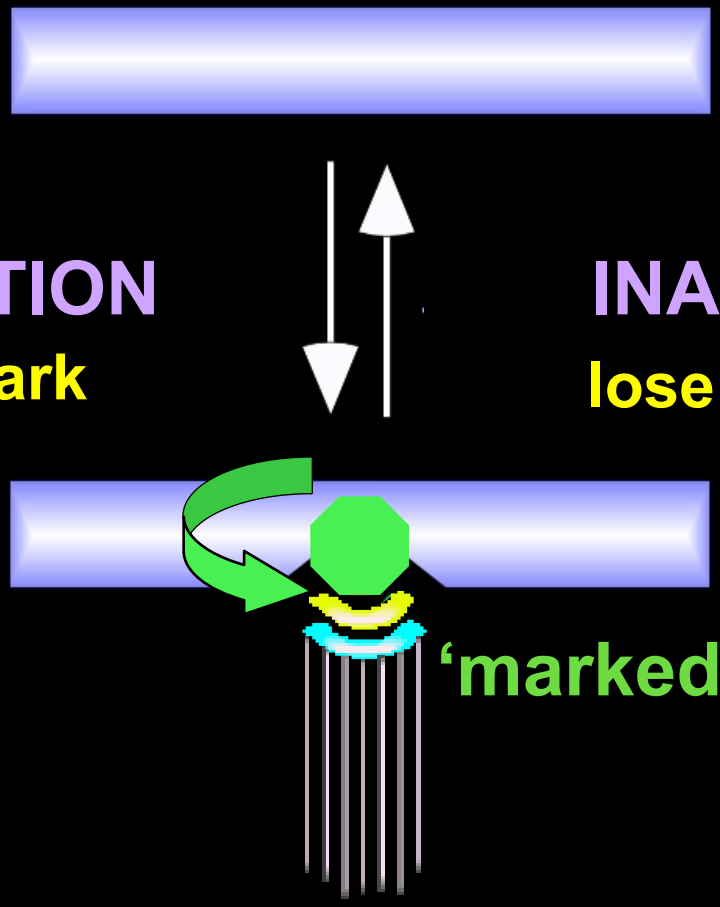




Epigenetic Model for CEN Identity

primary sequence is not sufficient (dicentrics)

non-centromeric sequence can acquire and propagate centromere function (neocentromeres)



ACTIVATION

gain epigenetic mark

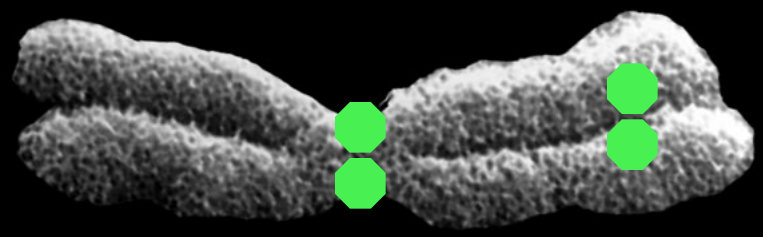
INACTIVATION

lose epigenetic mark

‘marked’ DNA/chromatin

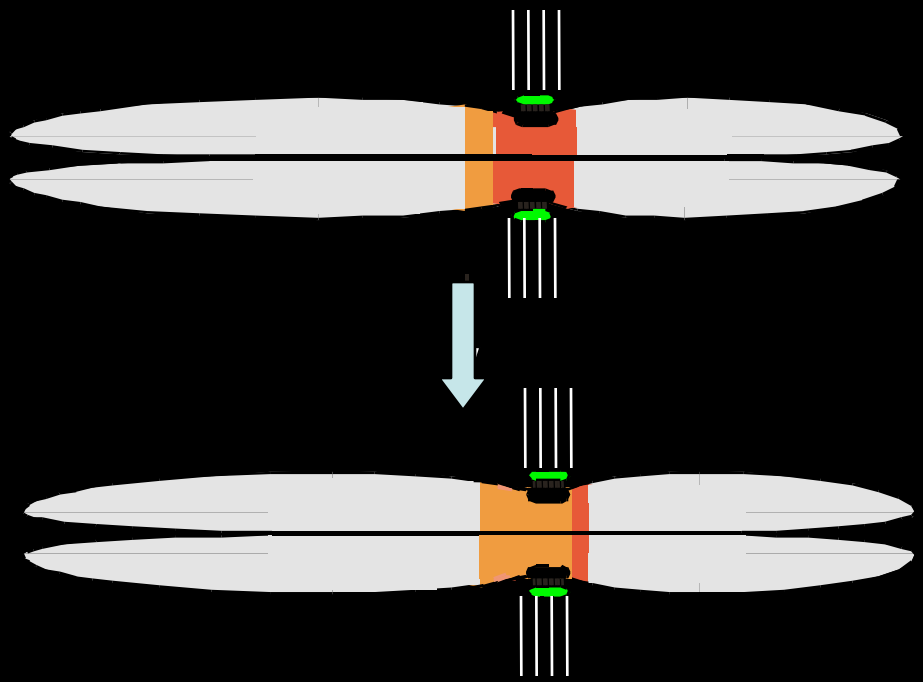
CEN Evolution: Hopping

in terms of individual cell cycles, cerevisiae sequence specificity makes the most sense



Centromere Plasticity Necessary for Chromosome Evolution ?

Satellite expansion & contraction: CEN spreading & hopping



Translocations: CEN inactivation & NeoCEN activation