Lecture 5
Chromosomes in the Cell Cycle

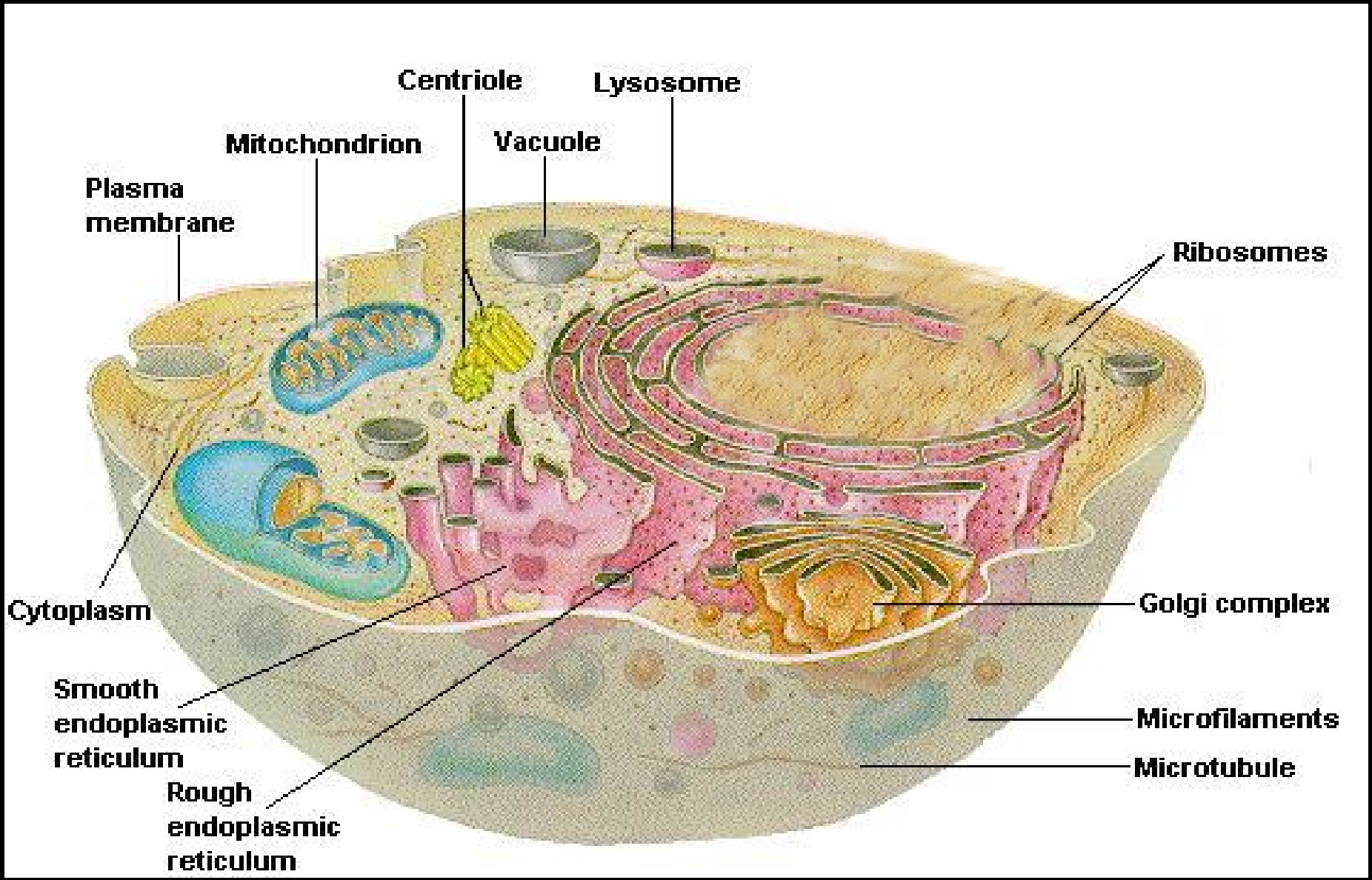
Outline:

Basics of Chromosome Organization

Chromosome Organization and Function in Interphase

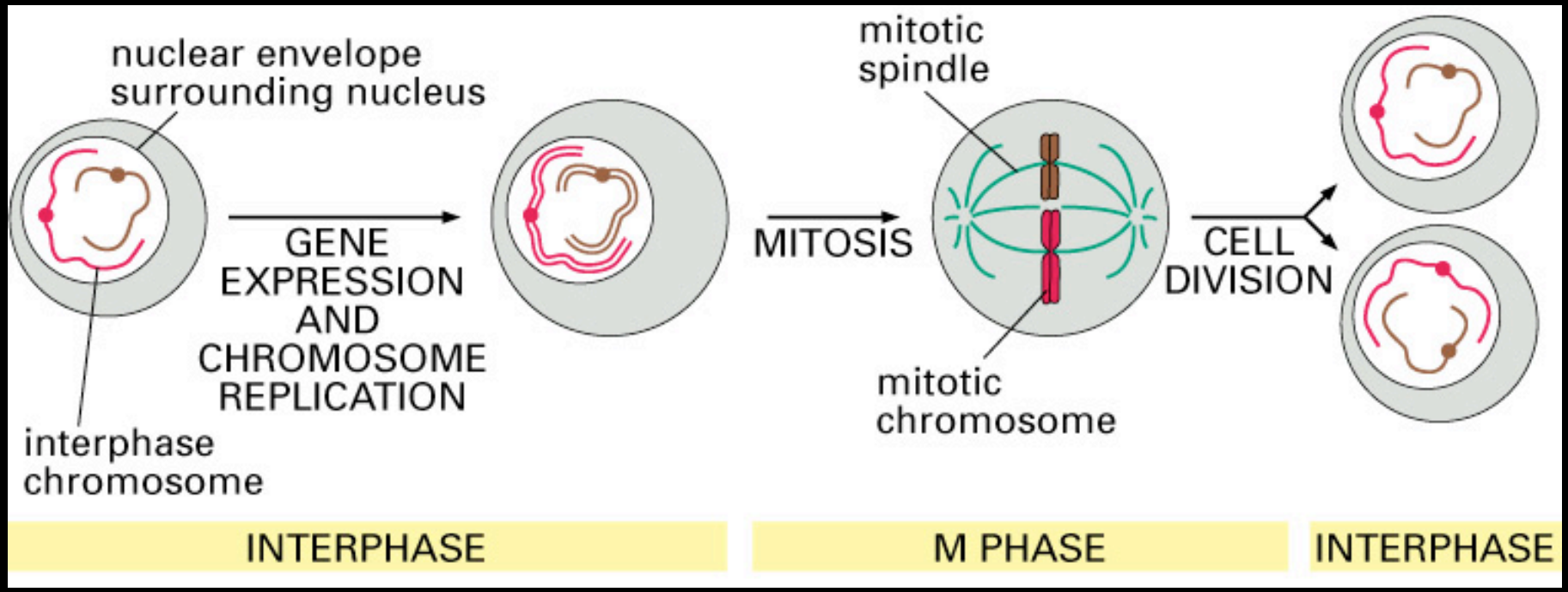
Paper:

Some Cell Biologists view of the Cell....

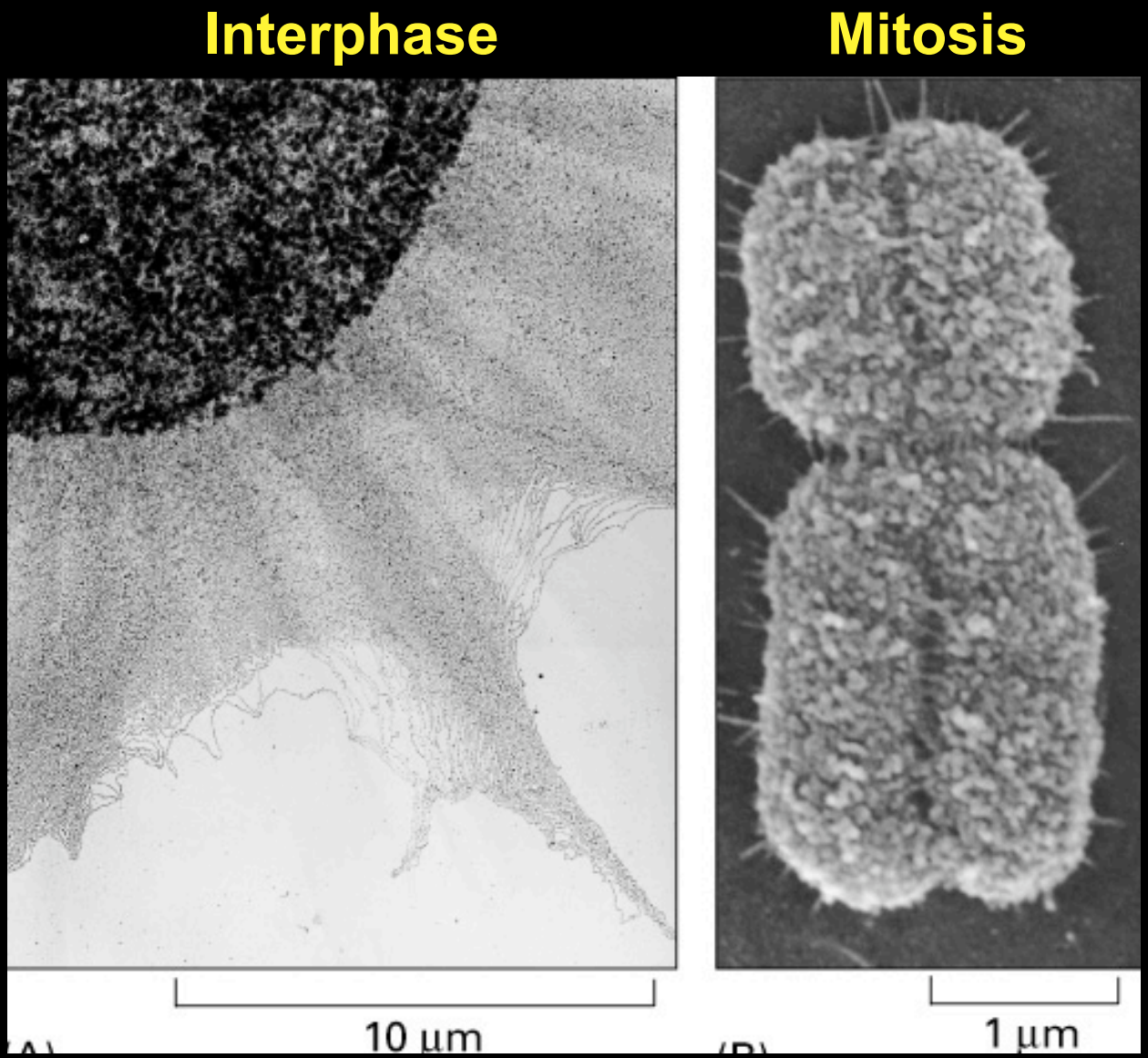


the nucleus **IS** part of the cell and cell biology

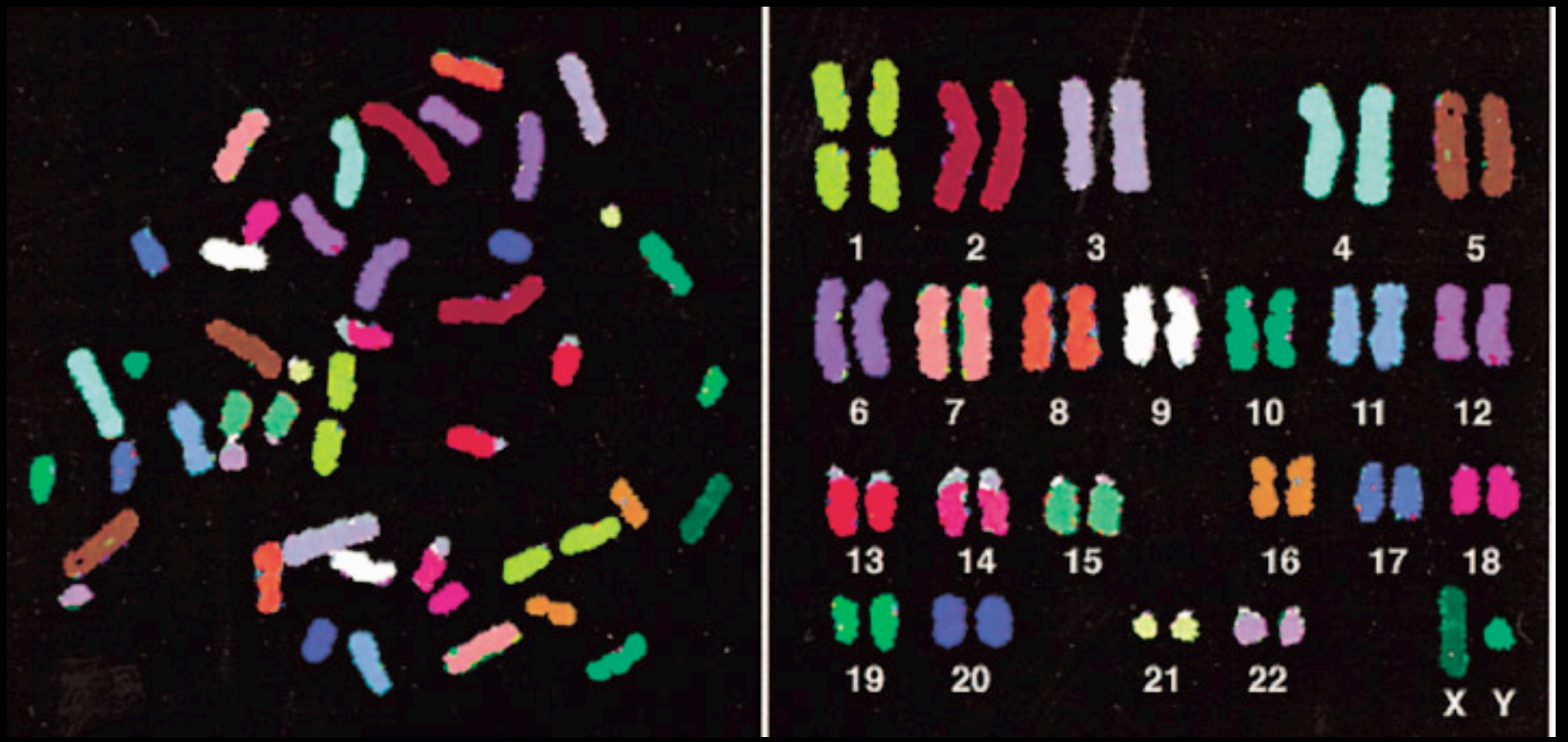
Chromosome Organization Changes Dramatically during the Cell Cycle



Chromosome Organization Changes Dramatically during the Cell Cycle



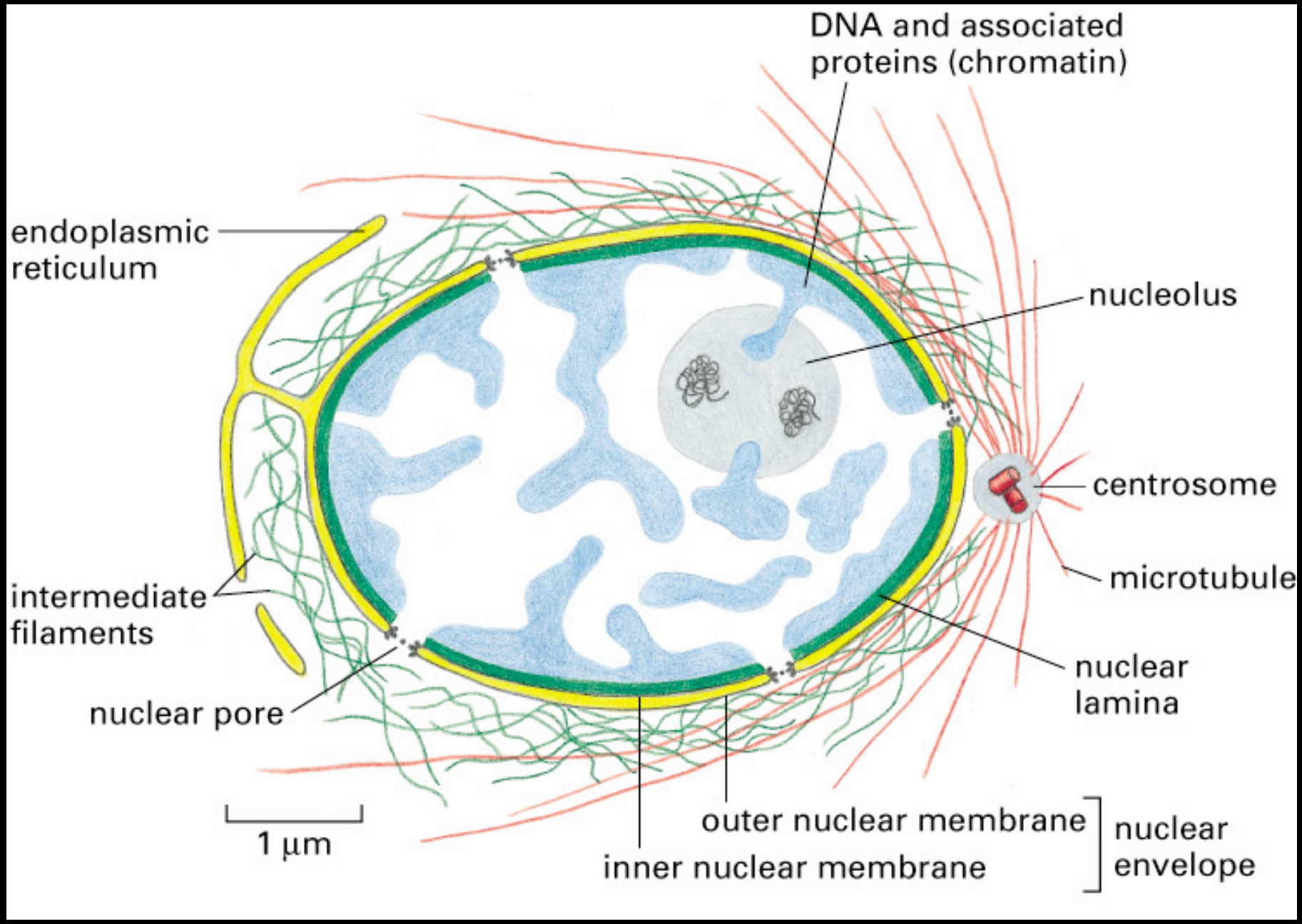
Humans mitotic chromosomes are colorful



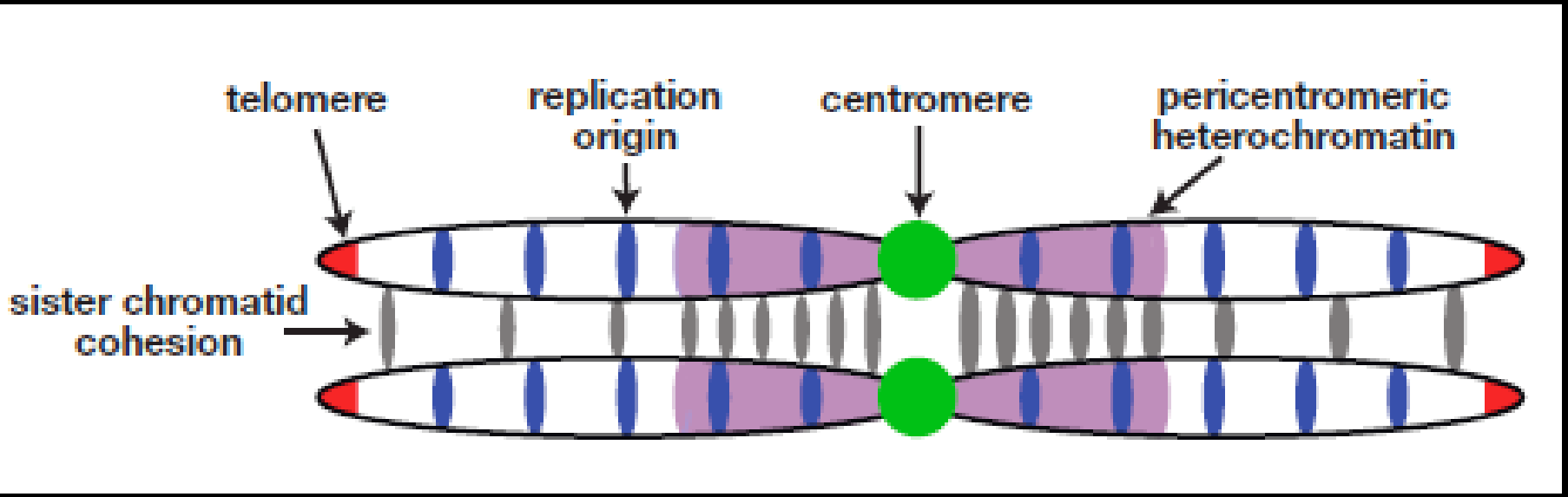
SKY-Spectral KarYotyping

FISH with probes from FACs-sorted chromosomes each labeled with unique mixture of fluorophores

Organization of DNA in the Nucleus



Organization of Eukaryotic Chromosomes/Genomes



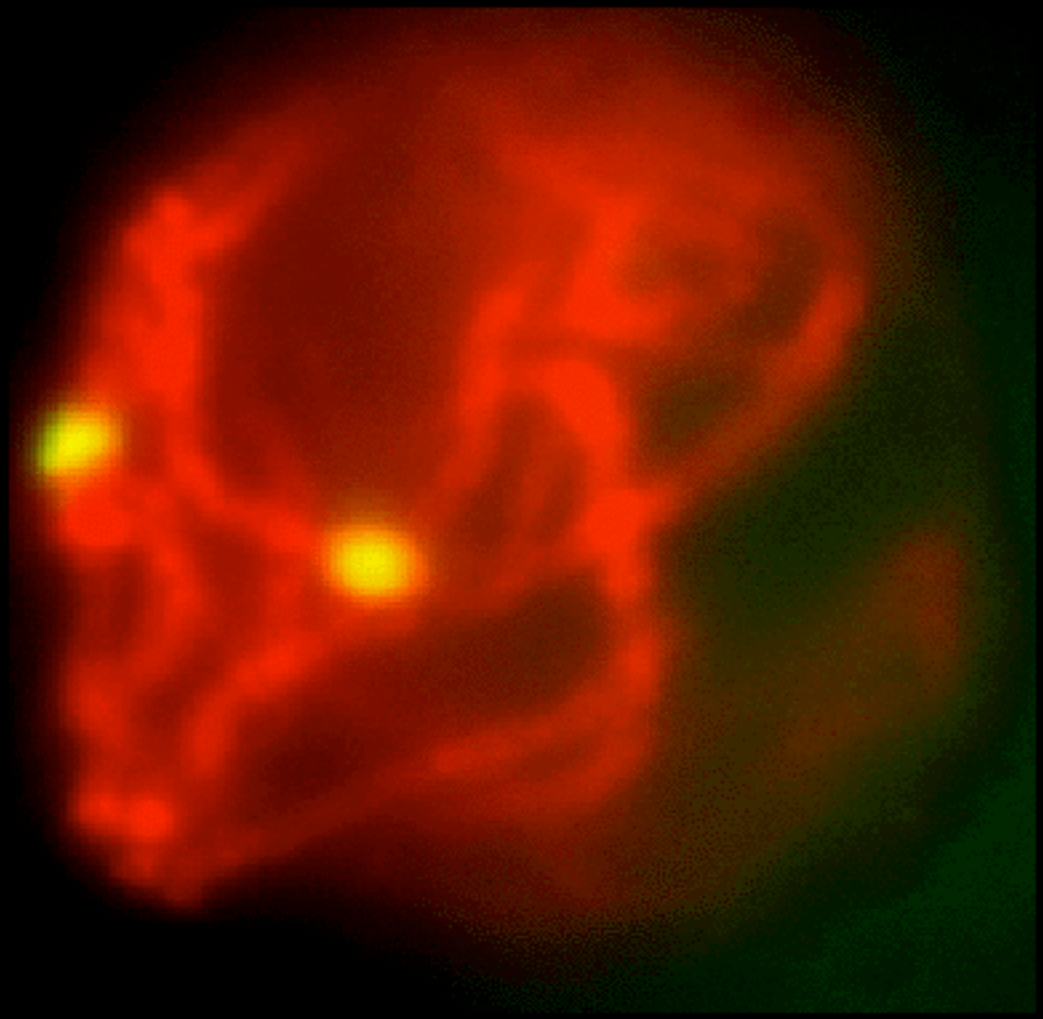
heterochromatin

mostly repeated DNA
some single copy genes
condensed through cell cycle?
stains brightly with DAPI

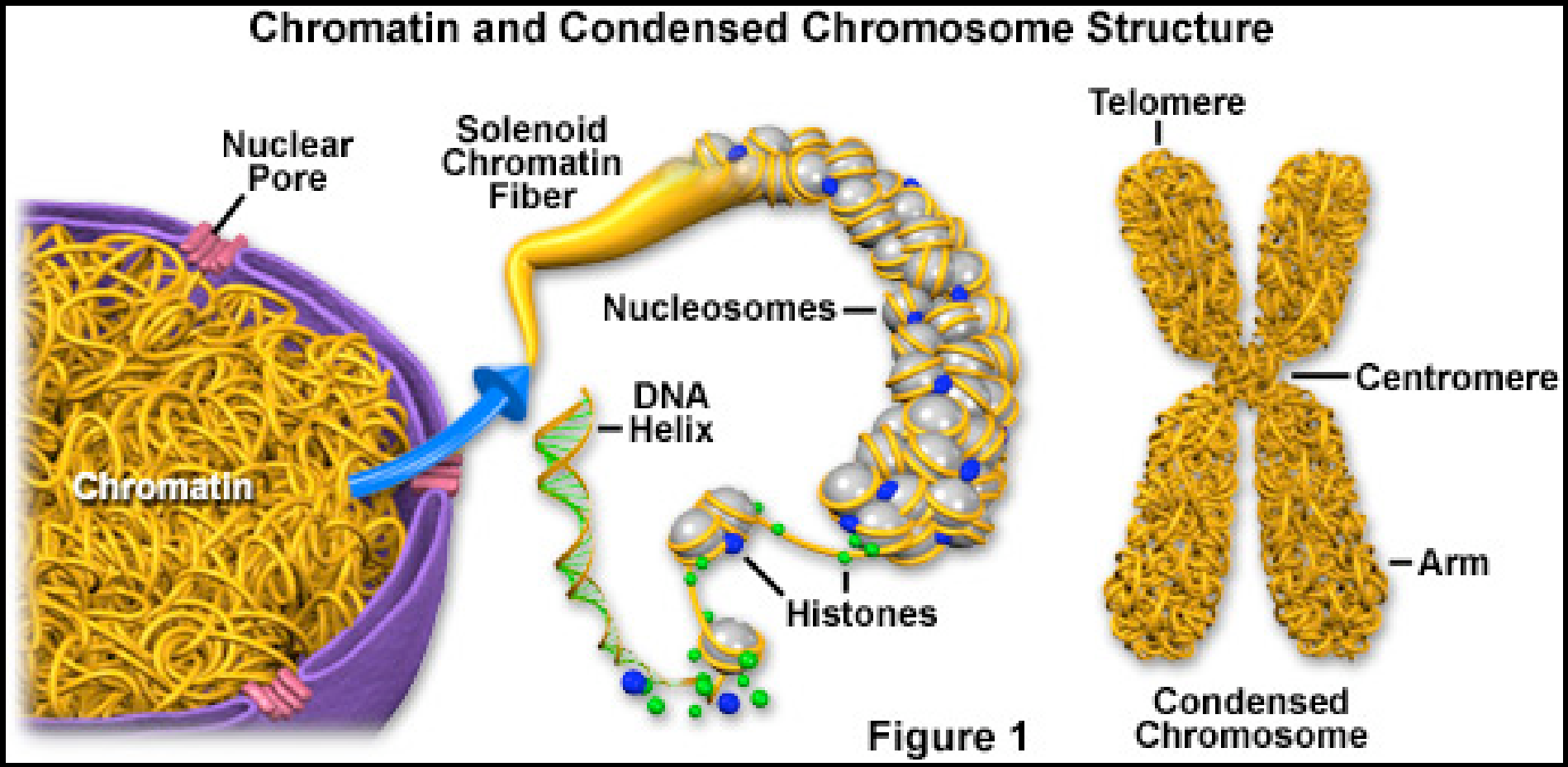
euchromatin

mostly single copy DNA
repeat content depends on organism
condensed/decondensed through cycle
stains weakly with DAPI

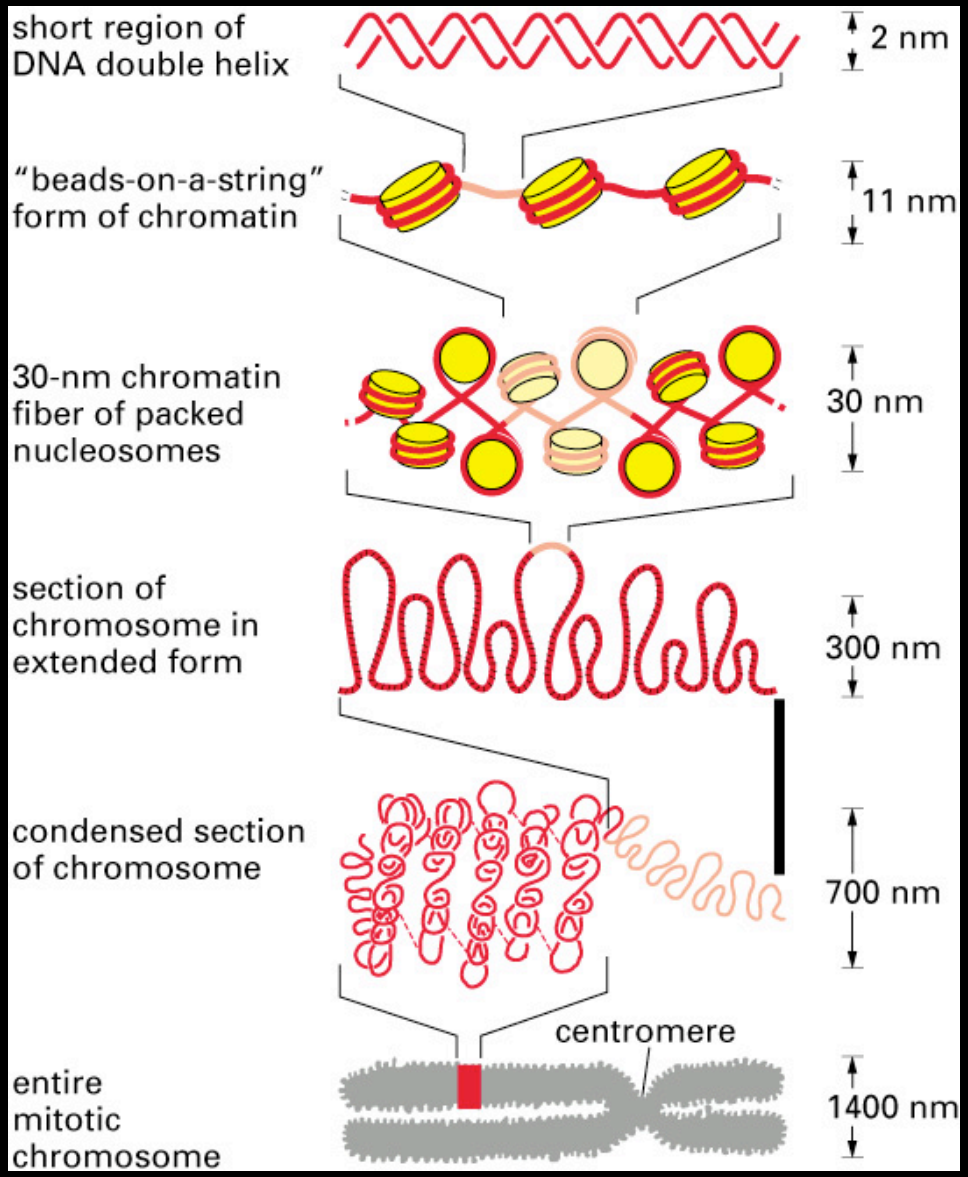
Heterochromatic ‘Knobs’ in Corn



many levels of packaging



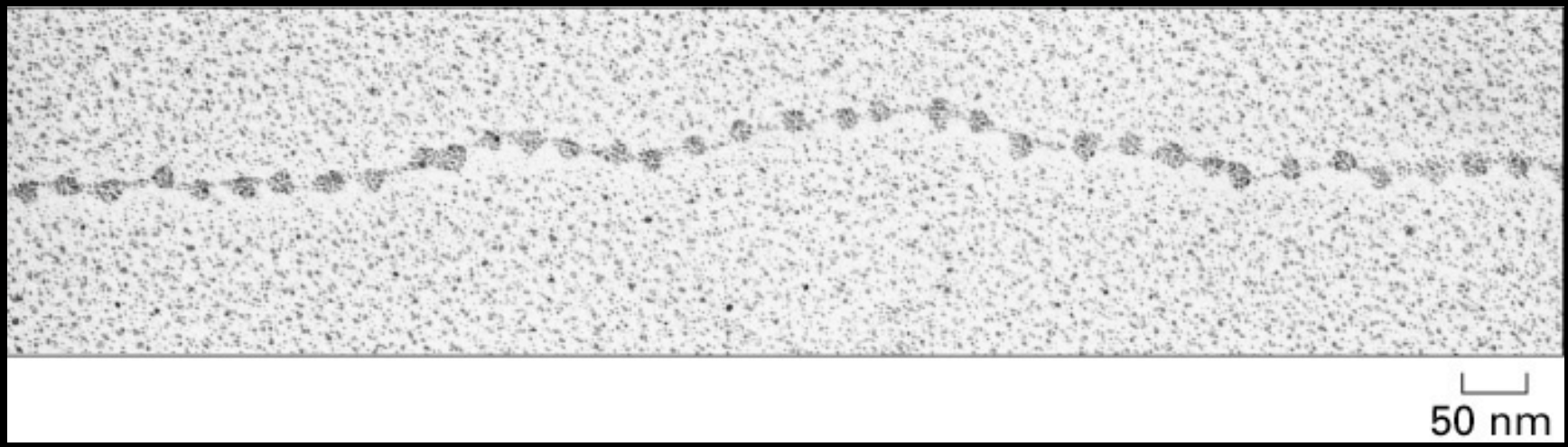
many levels of packaging



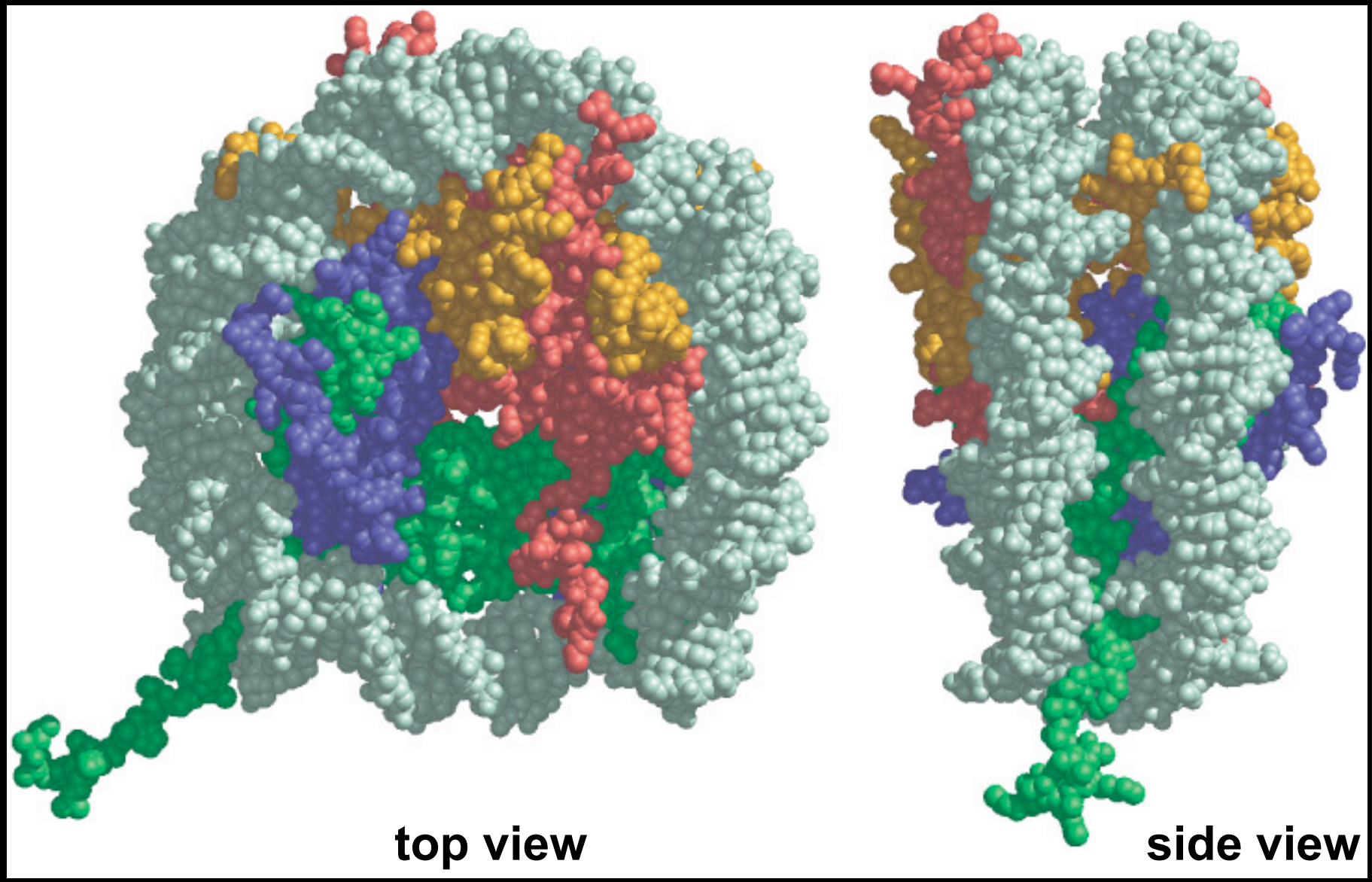
mitotic chromosome - 10,000 fold shorter than length of DNA molecules

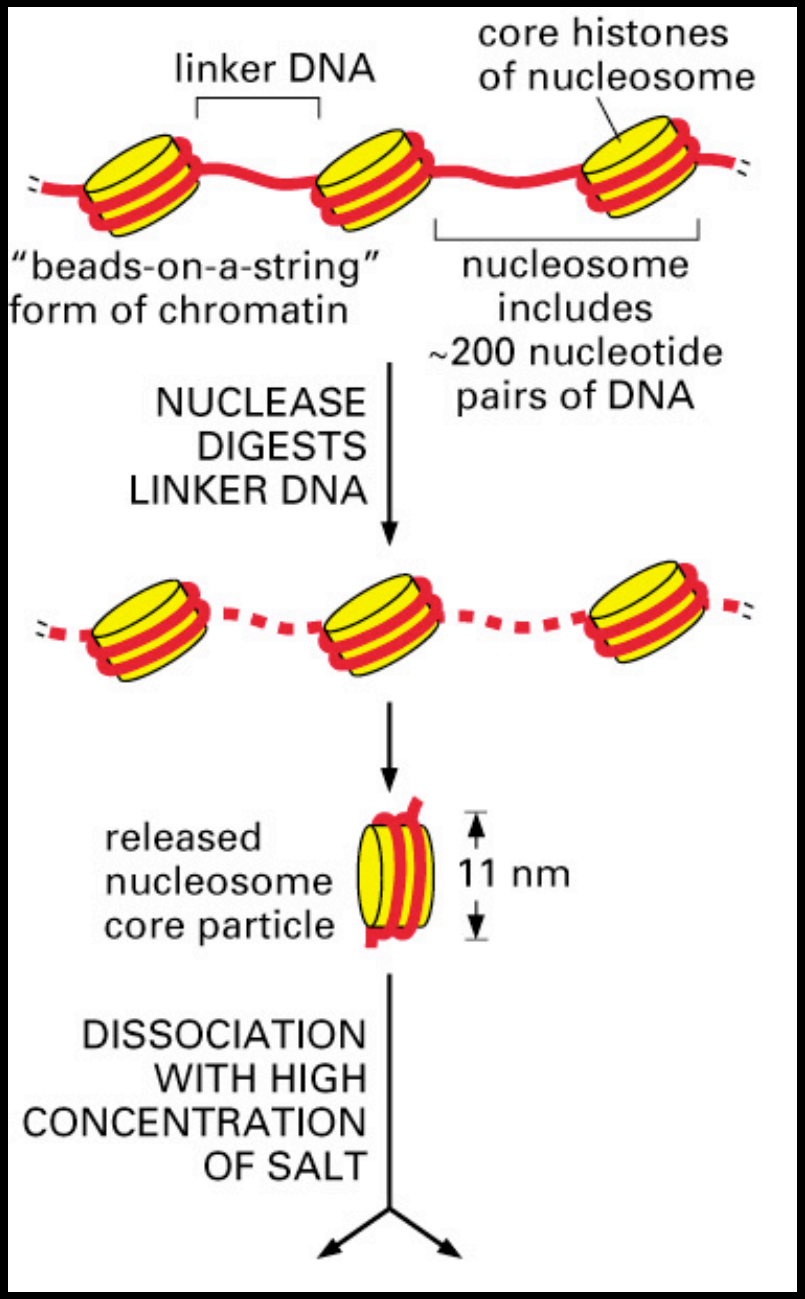
‘beads on a string’ = nucleosomes

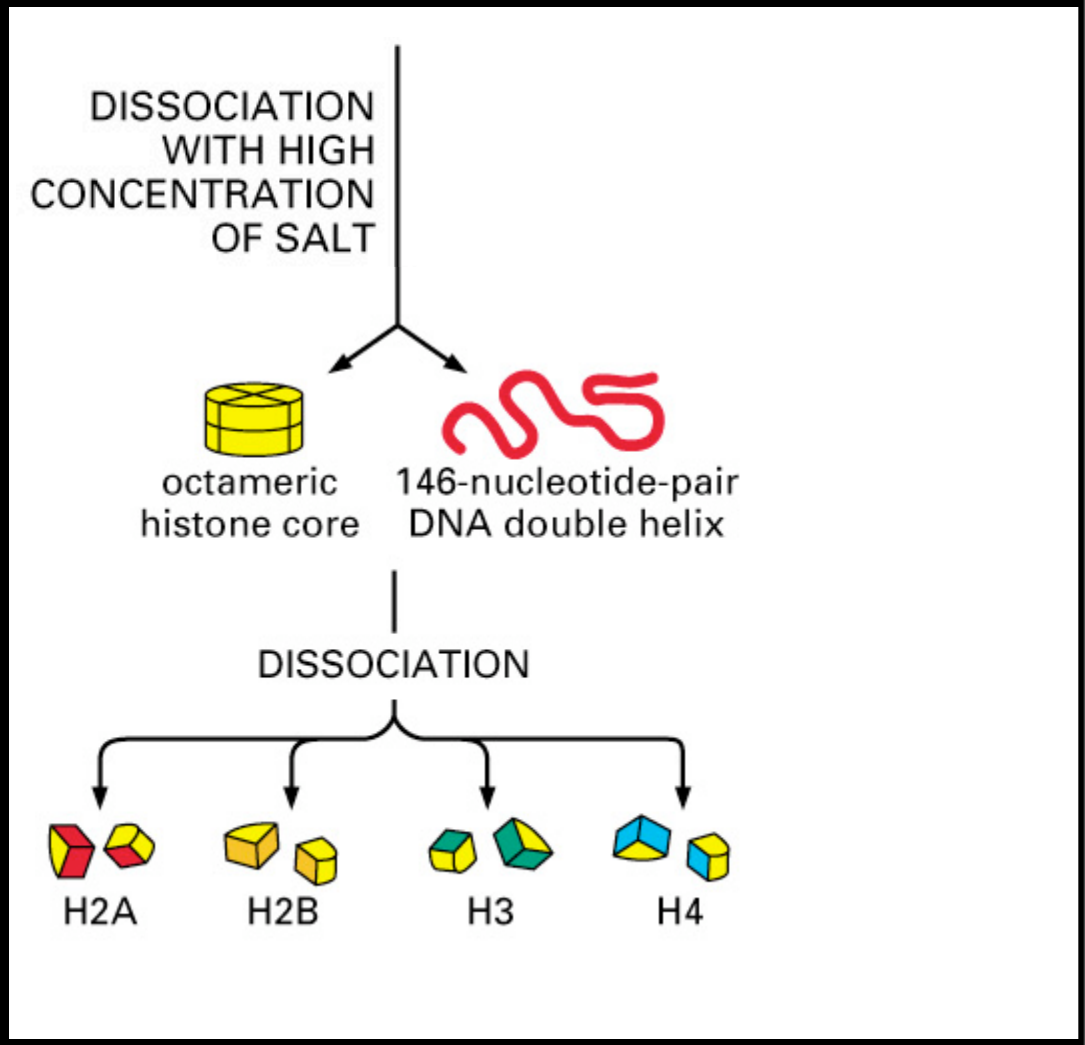
low salt treatment to remove ‘weakly bound’ proteins



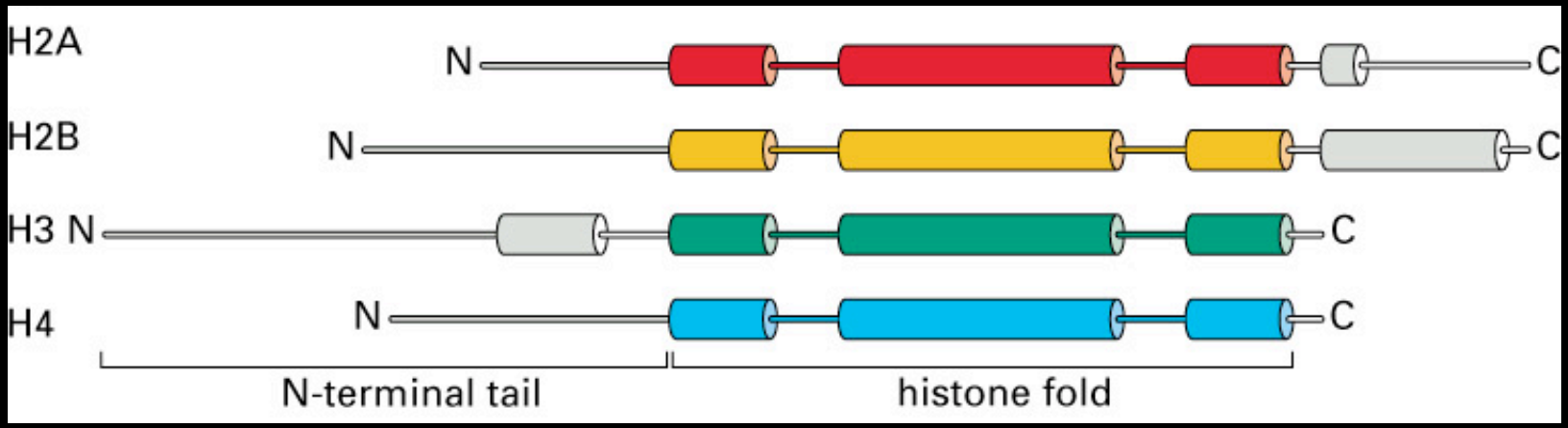
‘beads’ = core histones, ‘strings’ = DNA

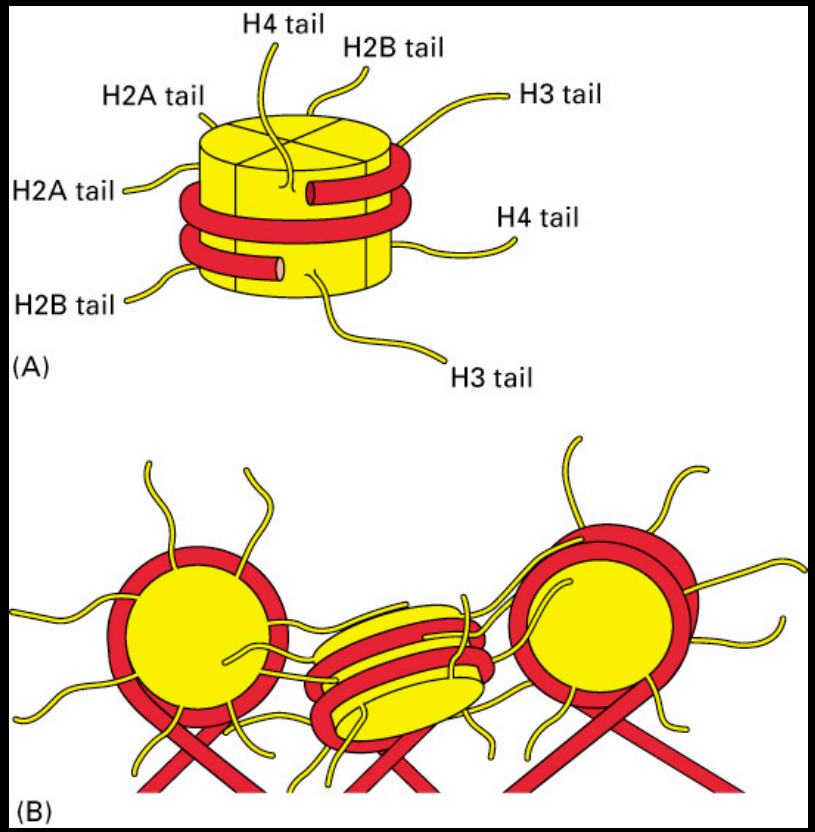



linker DNA
core histones of nucleosome
"beads-on-a-string" form of chromatin
nucleosome includes ~200 nucleotide pairs of DNA
NUCLEASE DIGESTS LINKER DNA
released nucleosome core particle
11 nm
DISSOCIATION WITH HIGH CONCENTRATION OF SALT

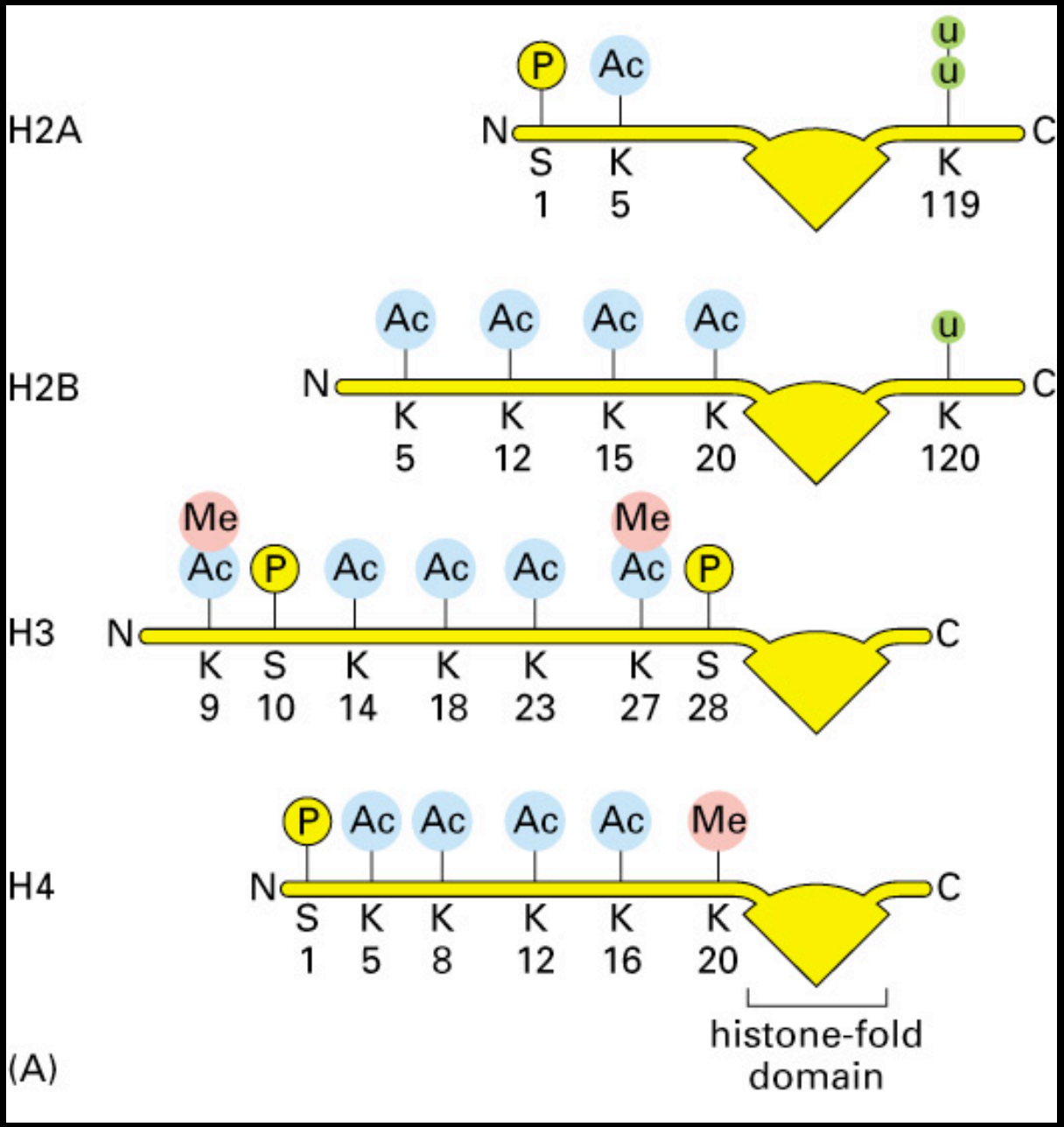

DISSOCIATION WITH HIGH CONCENTRATION OF SALT
octameric histone core
146-nucleotide-pair DNA double helix
DISSOCIATION
H2A
H2B
H3
H4

Histone N-terminal tails.....NOT in the crystal structure

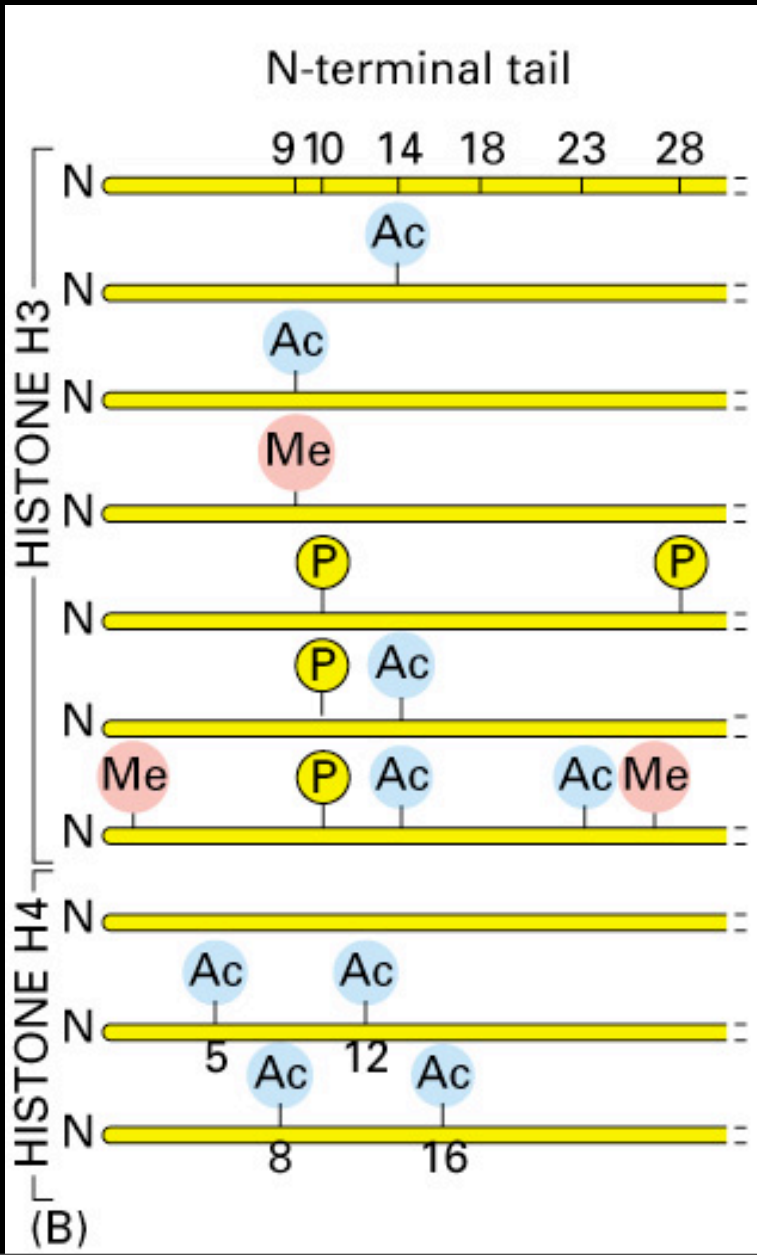




Histones tails (and cores) are post-translationally modified



Histone modifications are correlated with different properties (functions?)



modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?
unmodified	gene silencing?
acetylated	histone deposition
acetylated	gene expression

Proteins associated with Histone modifications

Enzymes:

methyltransferases (HMTases)

acetyltransferases (HATs)

deacetylases (HDACs)

demethylases

different enzymes for each modification

- Suvar3-9 - H3K9me
- Ez - H3K27me
- Trx - H3K4me

Binding Proteins:

Heterochromatin Protein 1 (HP1)

H3K9me2 and 3

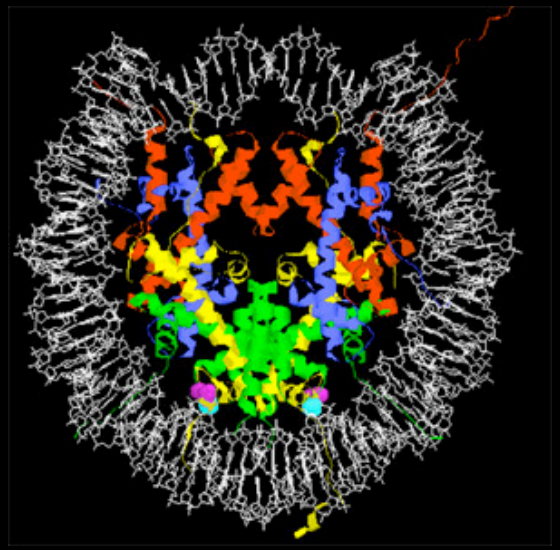
Polycomb Proteins (Pc)

H3K27me2 and 3

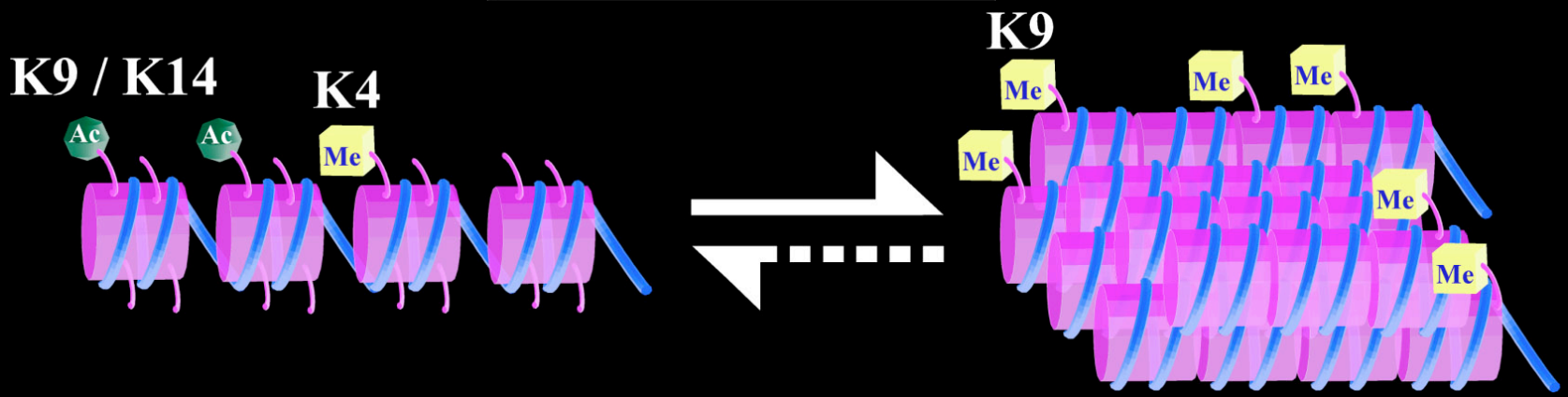
bind modification via ‘chromodomain’

The ‘Histone Code’ Hypothesis

D. Allis
T. Jenuwein



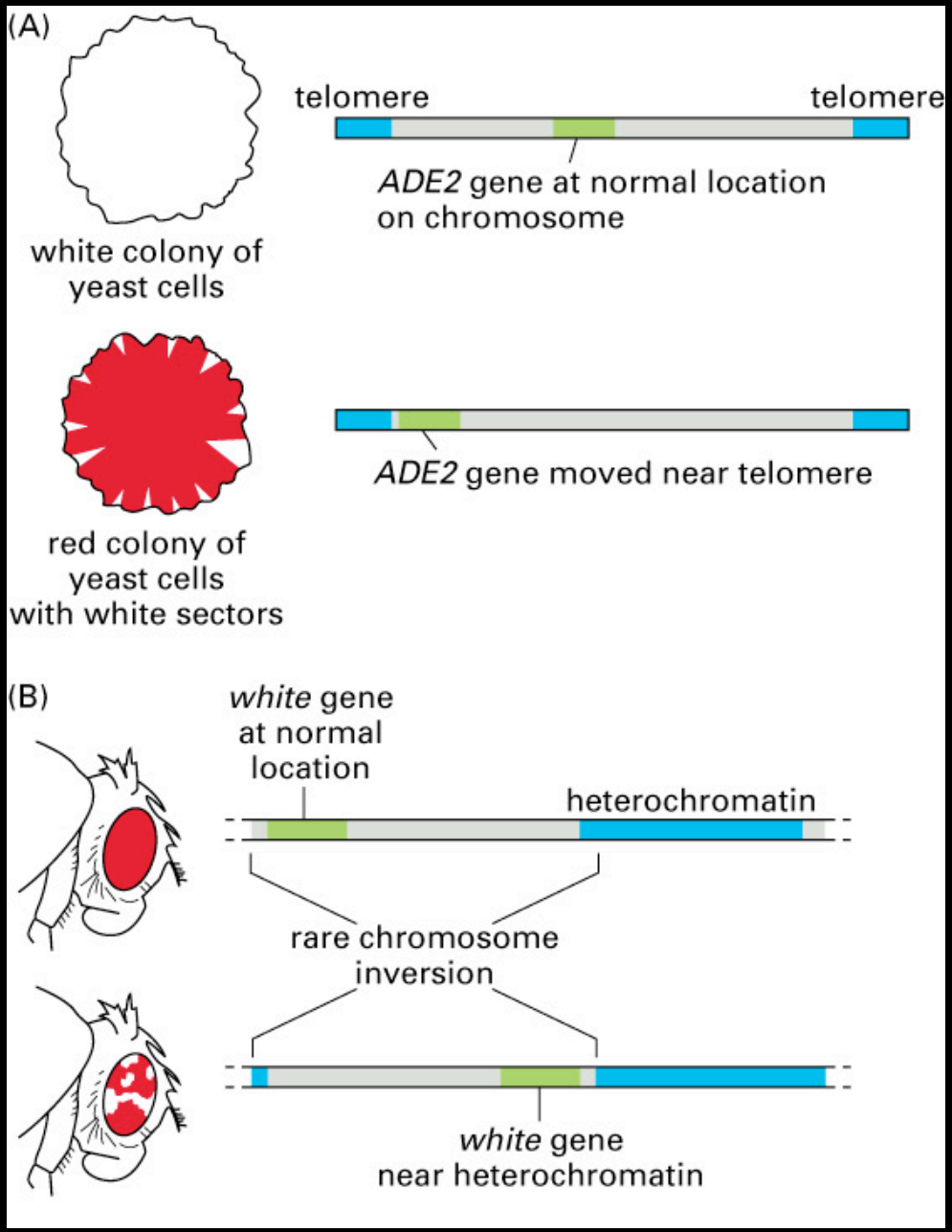
methylation
acetylation
phosphorylation
SUMOylation
ubiquitination



‘active’ or ‘open’ chromatin

‘silent’ chromatin

Position Effect Variegation (PEV) = Silencing



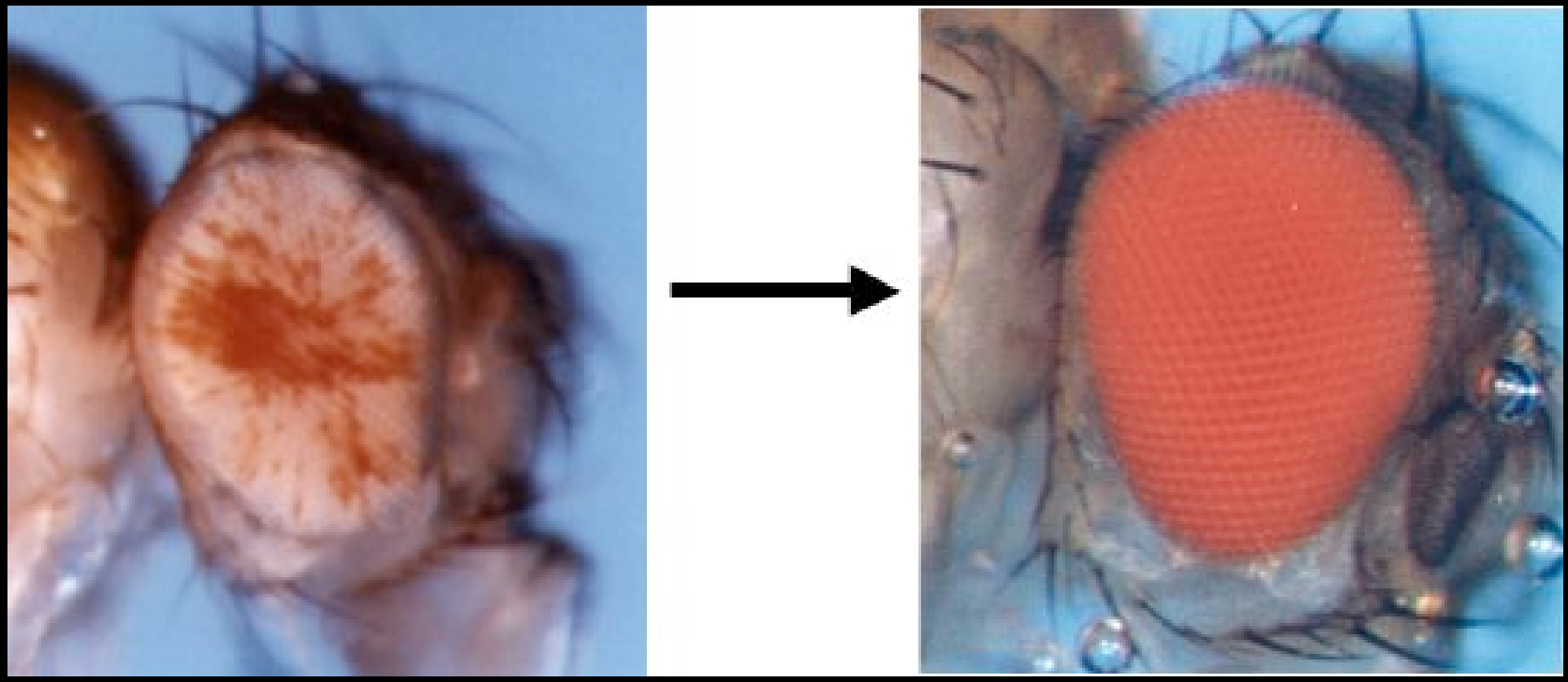
abnormal proximity
to heterochromatin causes
CLONAL, HERITABLE SILENCING
of normally active genes

able to spread
over Mbs of DNA

heritable change in function
without change in DNA sequence
(EPIGENETIC)

Mutations in Histone Modification Proteins Affect Silencing

Suppressors and Enhancers of Variegation (Su(vars) and E(vars)



Su(var)3-9 H3K9 HMTase
Su(var)2-5 HP1 K9me binding protein

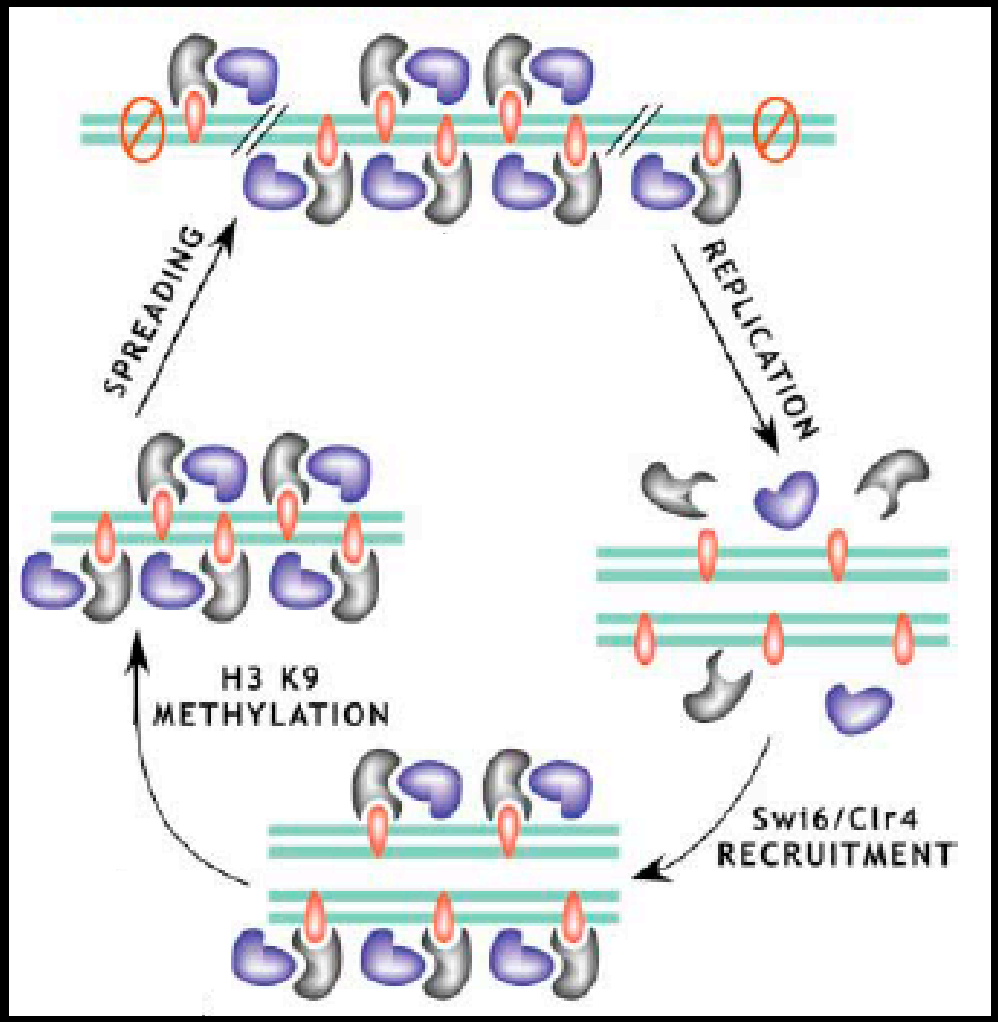
Epigenetic inheritance of functional states depends on histone modifications and associated proteins

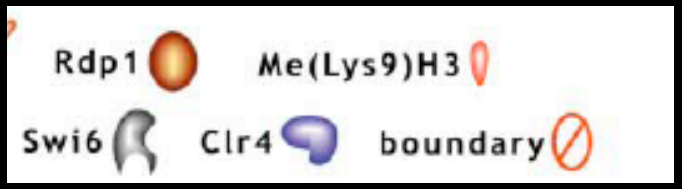
proteins act cooperatively to propagate and spread modification state

POMBE

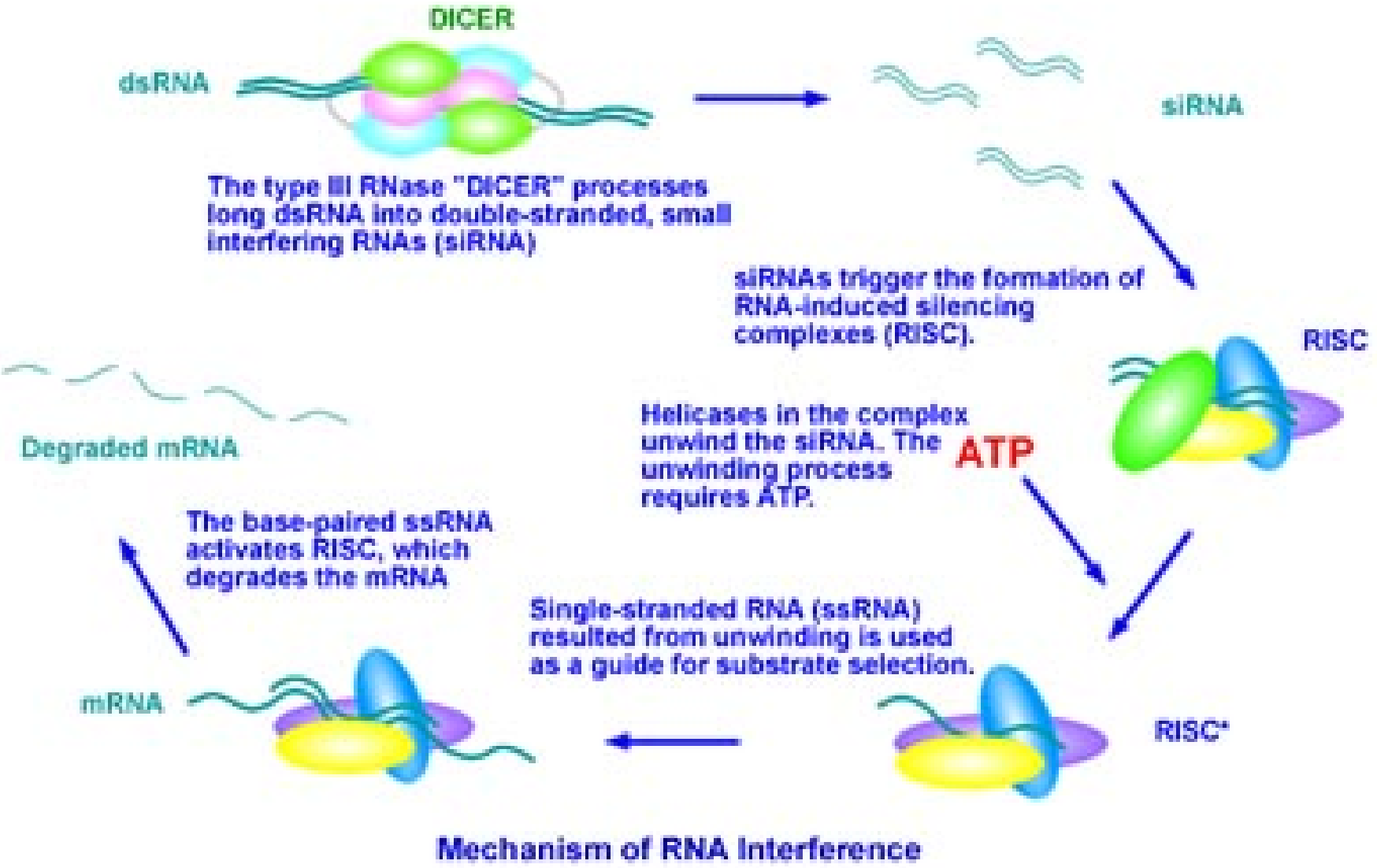
SWI6 = HP1 (binds H3K9me)

Clr4 = H3K9 HMTase



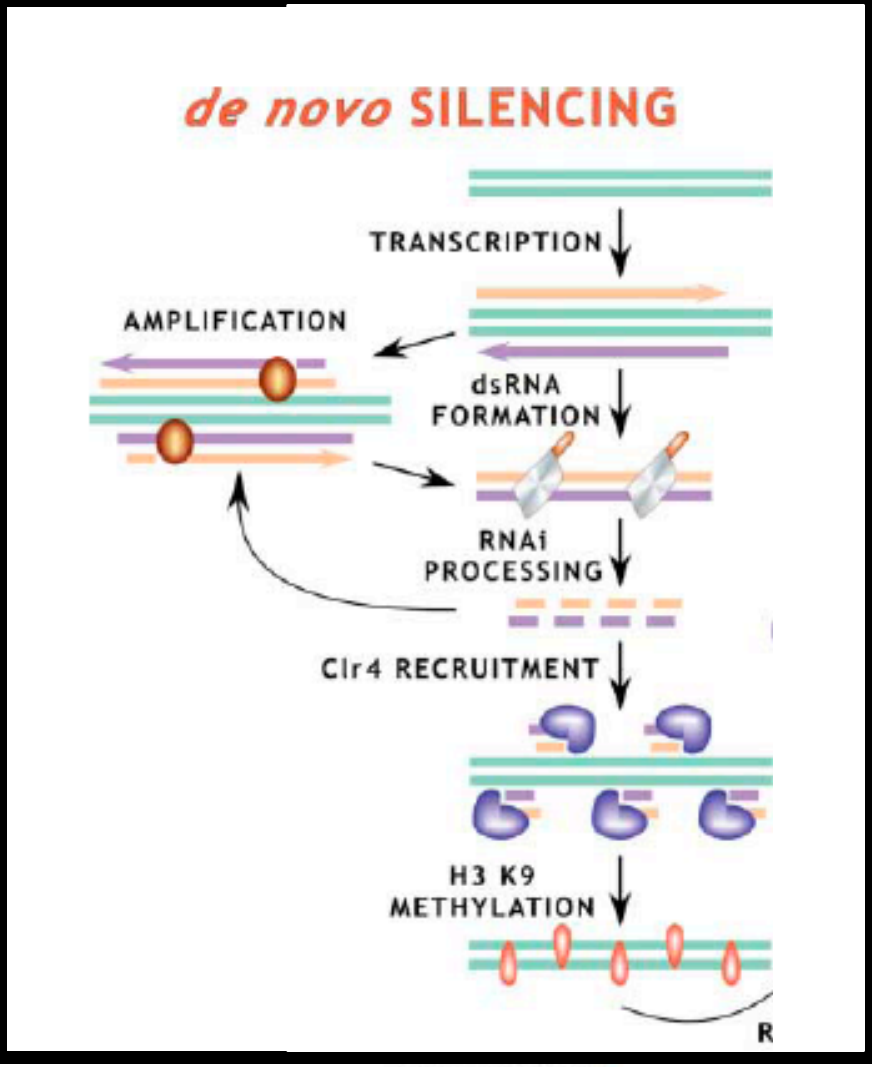


The RNAi Pathway and Post-transcriptional Silencing



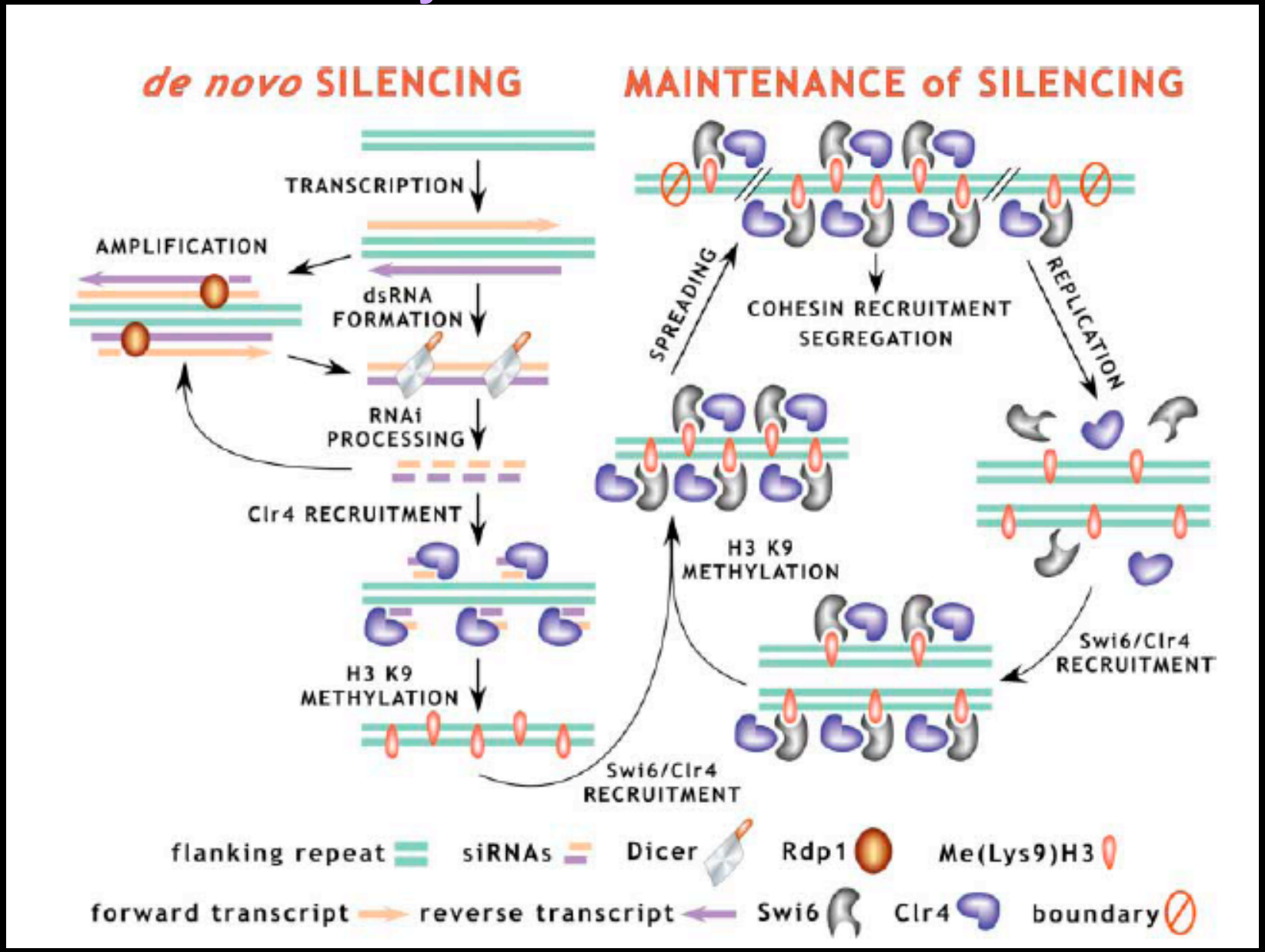
Mechanism of RNA Interference

The RNAi Pathway also Establishes Silent Chromatin

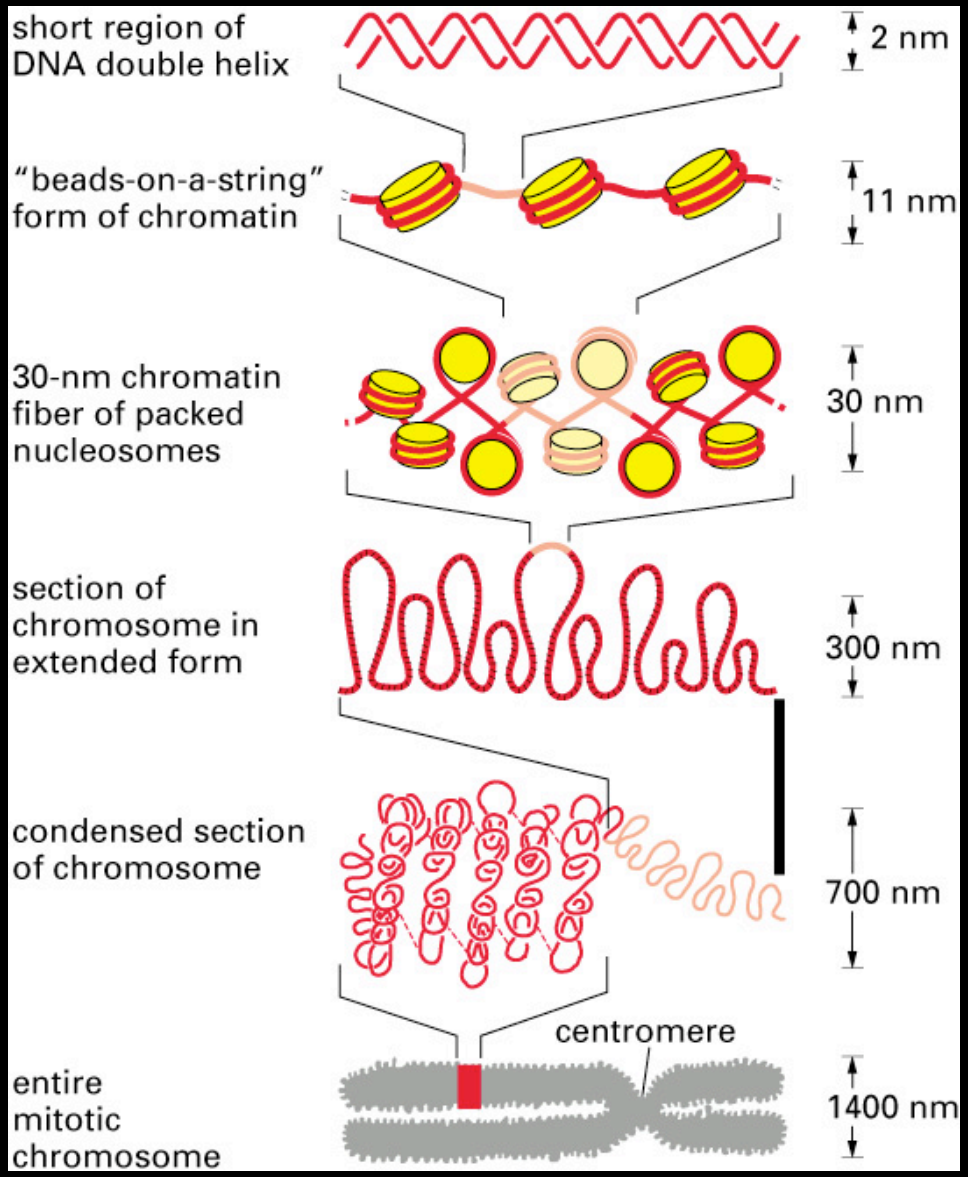


Grewal and others

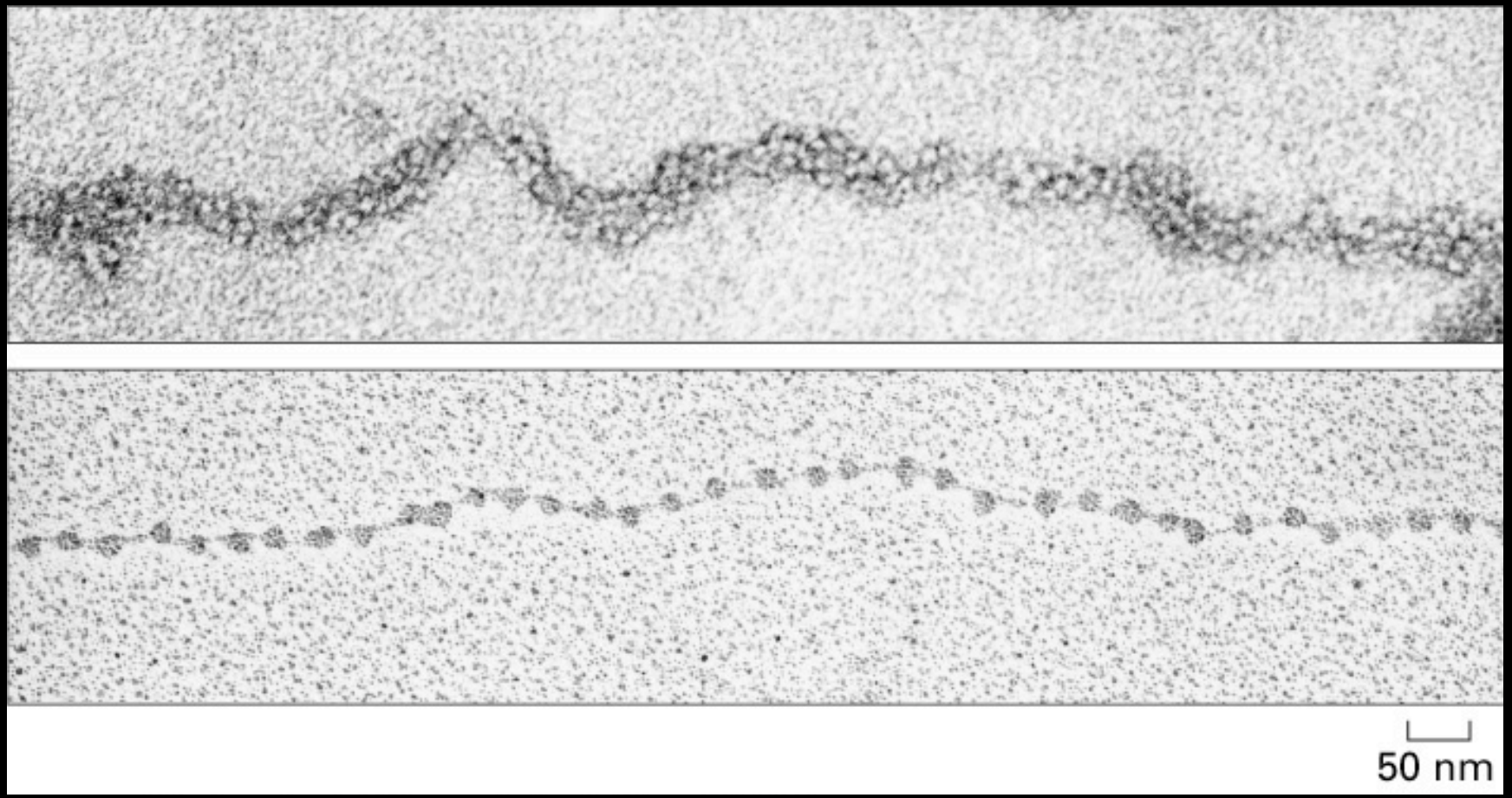
The RNAi Pathway also Establishes Silent Chromatin

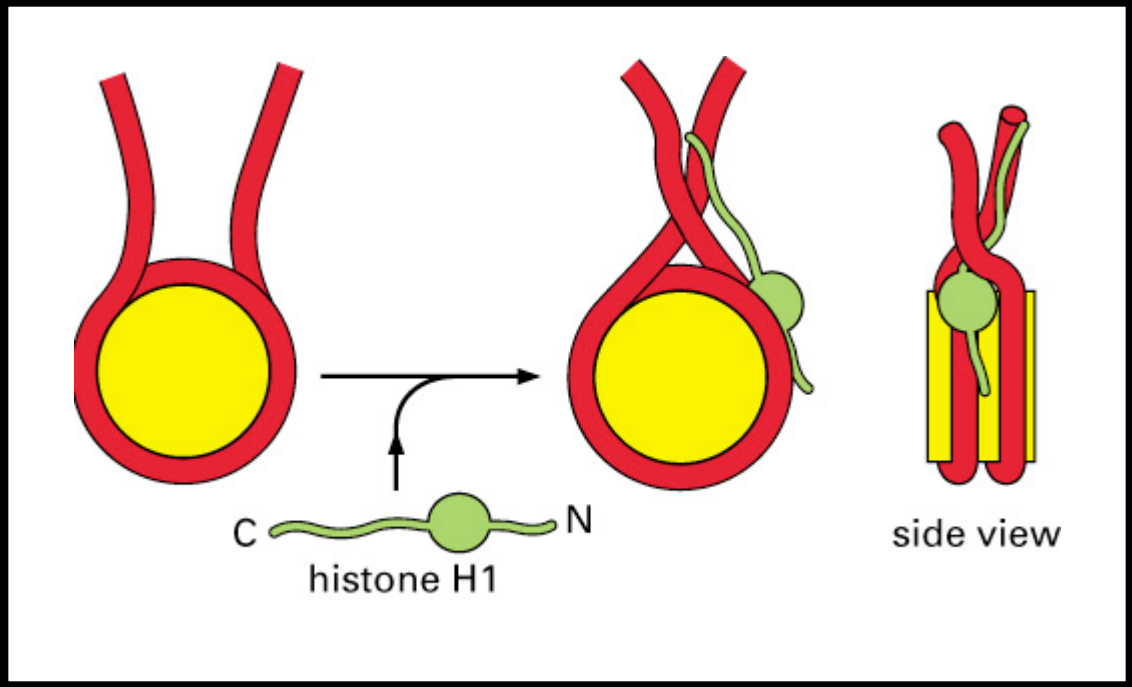


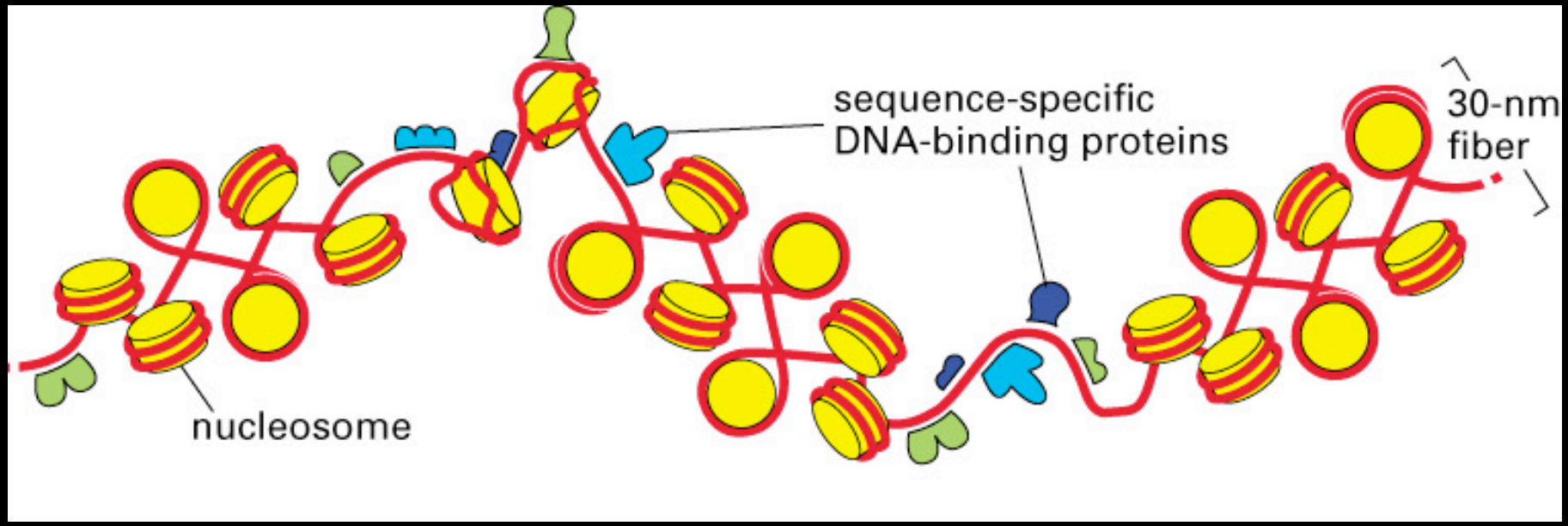
Grewal and others


short region of DNA double helix
2 nm
"beads-on-a-string" form of chromatin
11 nm
30-nm chromatin fiber of packed nucleosomes
30 nm
section of chromosome in extended form
300 nm
condensed section of chromosome
700 nm
centromere
entire mitotic chromosome
1400 nm

Beyond the beads....the 30nm fiber




C
N
histone H1
side view


sequence-specific
DNA-binding proteins
30-nm
fiber
nucleosome

Chromosome Organization and Function in Interphase

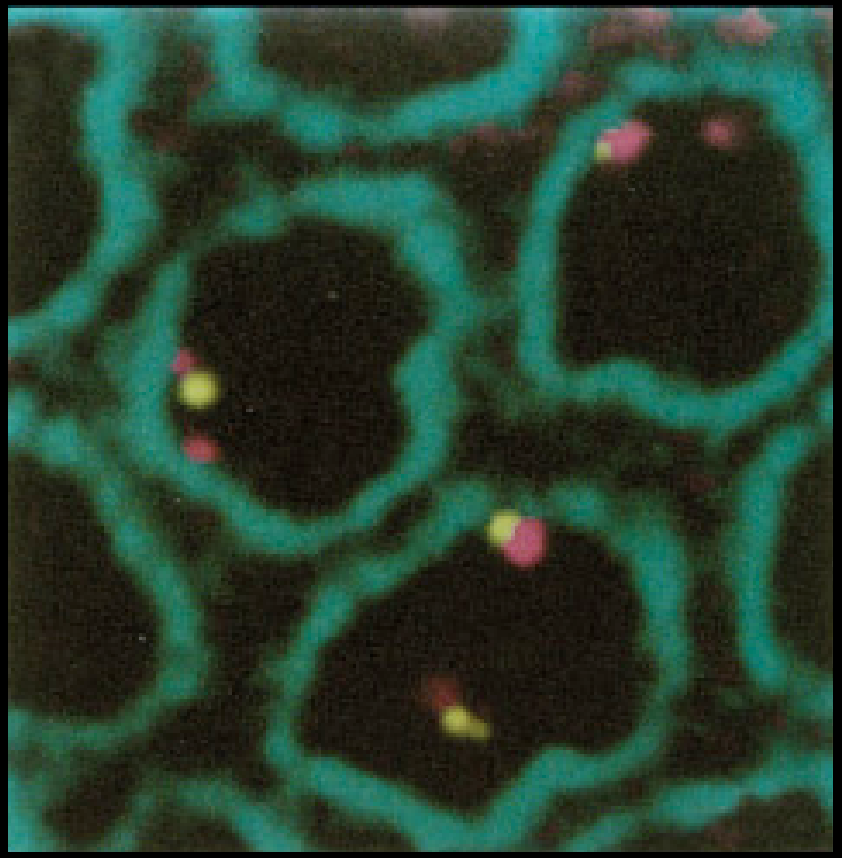
chromosomes are NOT randomly positioned in interphase

telomeres

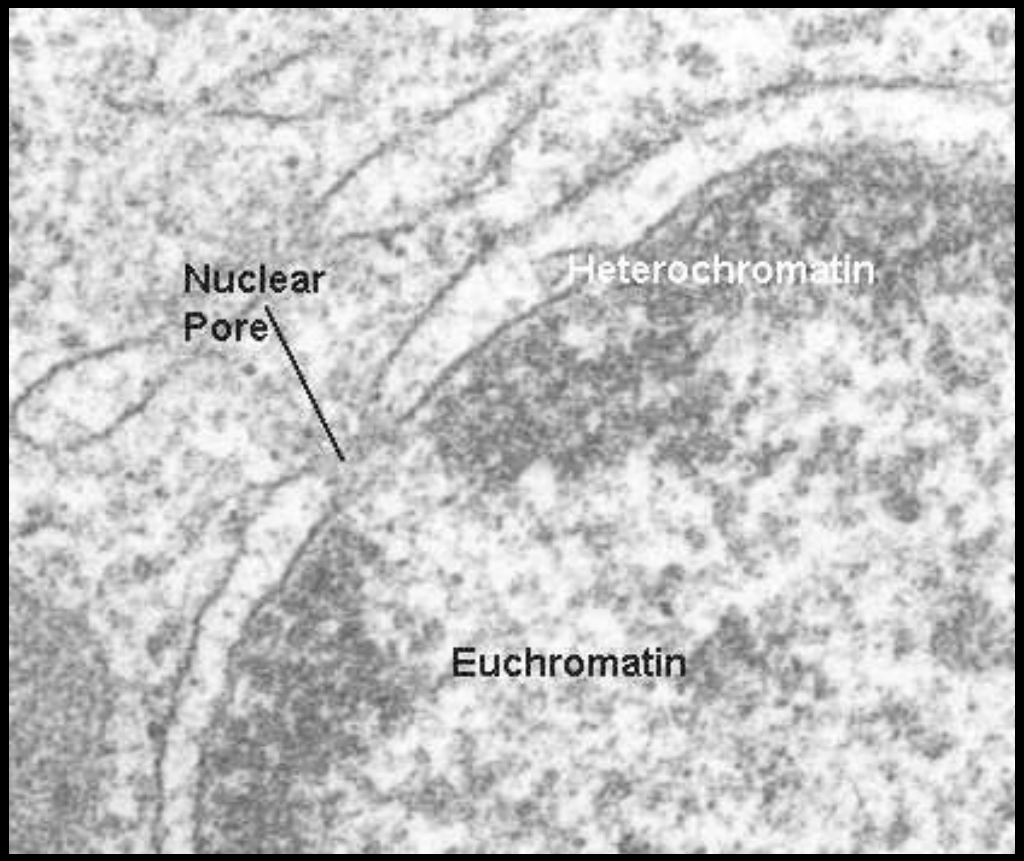
centromeres

Rabl configuration

Specific Regions are Associated with the Periphery

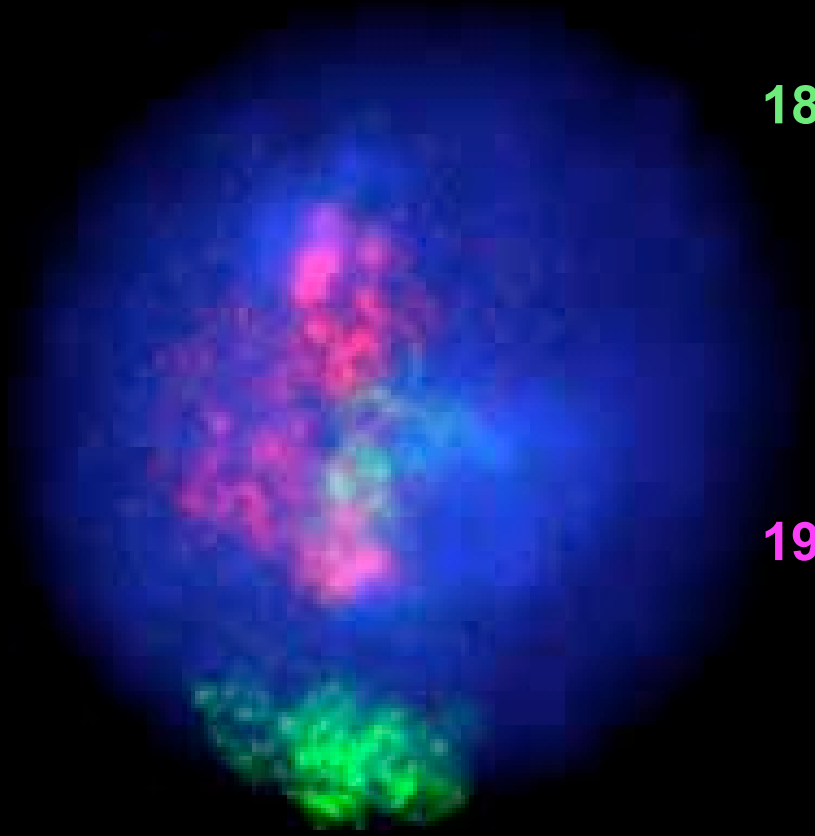


Telomeres
Lamin



Heterochromatin

Individual Chromosomes are Organized as Domains or Territories

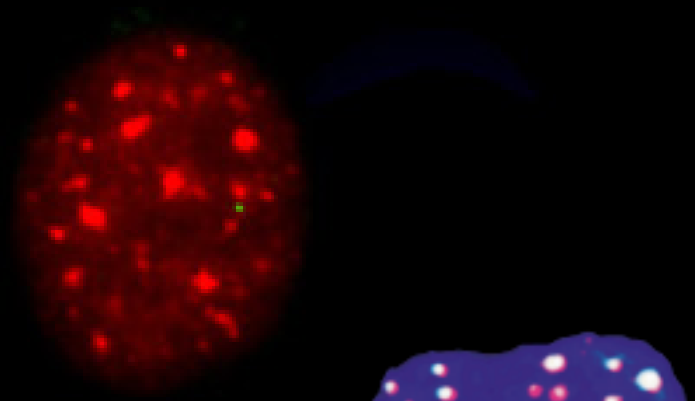


18 early replicating lots of genes

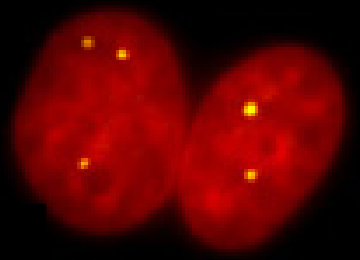
19 late replicating lower gene density

Bickmore, Cremer

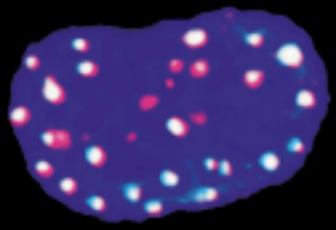
Order in the Interphase Nucleus: Nuclear 'Organelles' or 'Protein Bodies'



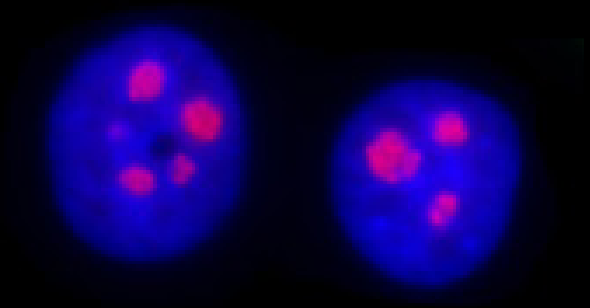
Replication 'factories'



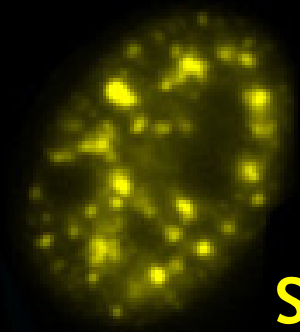
Cajal bodies (tx, processing)



PML bodies (tx)

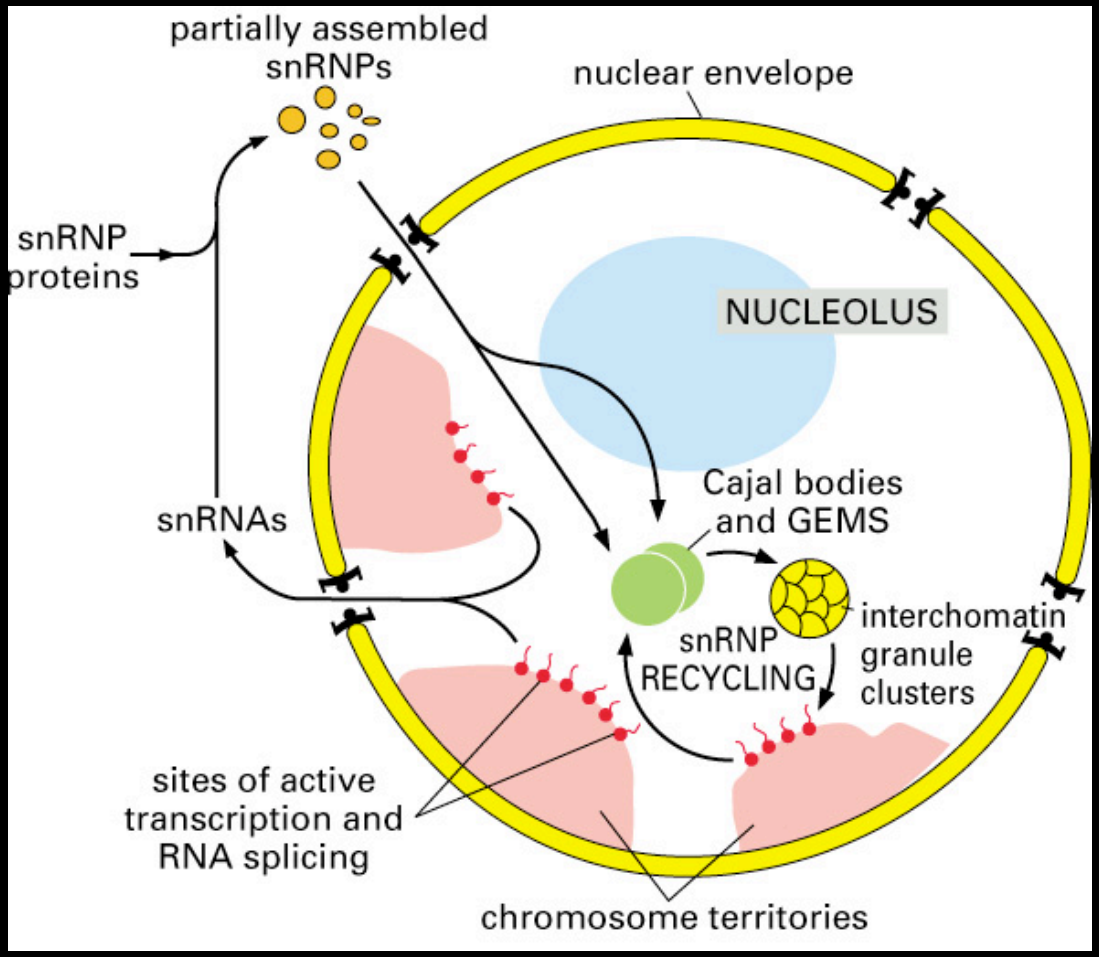


Nucleolus (rRNA tx and processing)



Speckles (splicing)

The Cajal Body

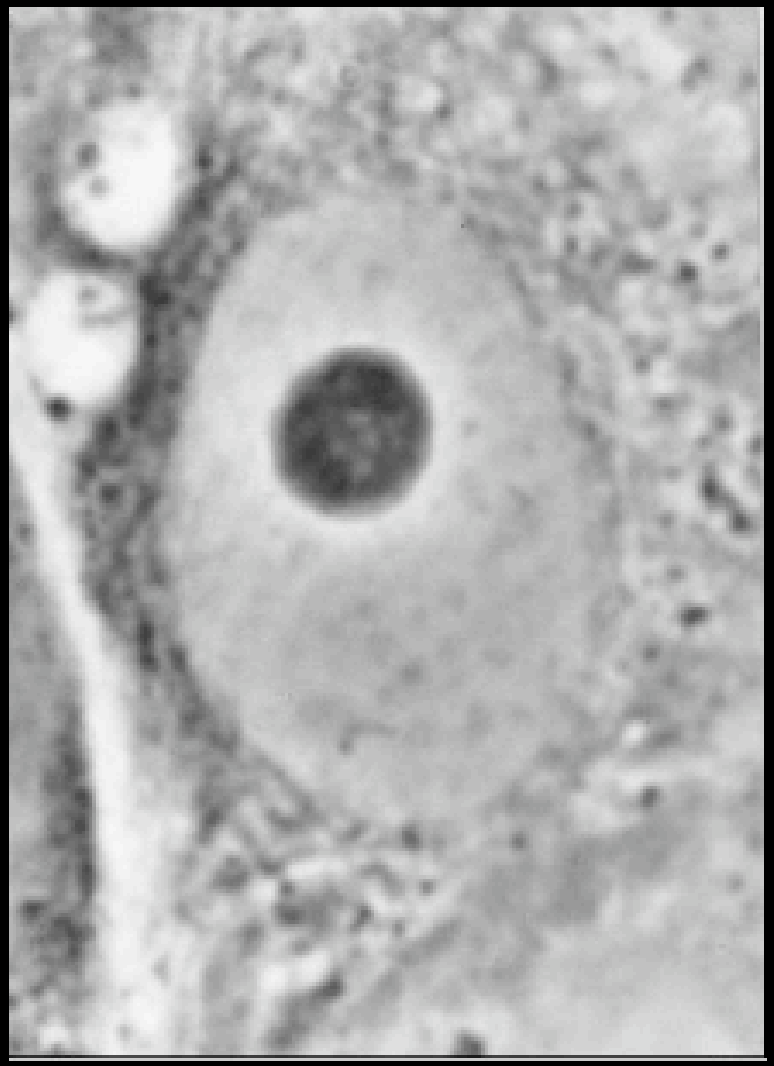


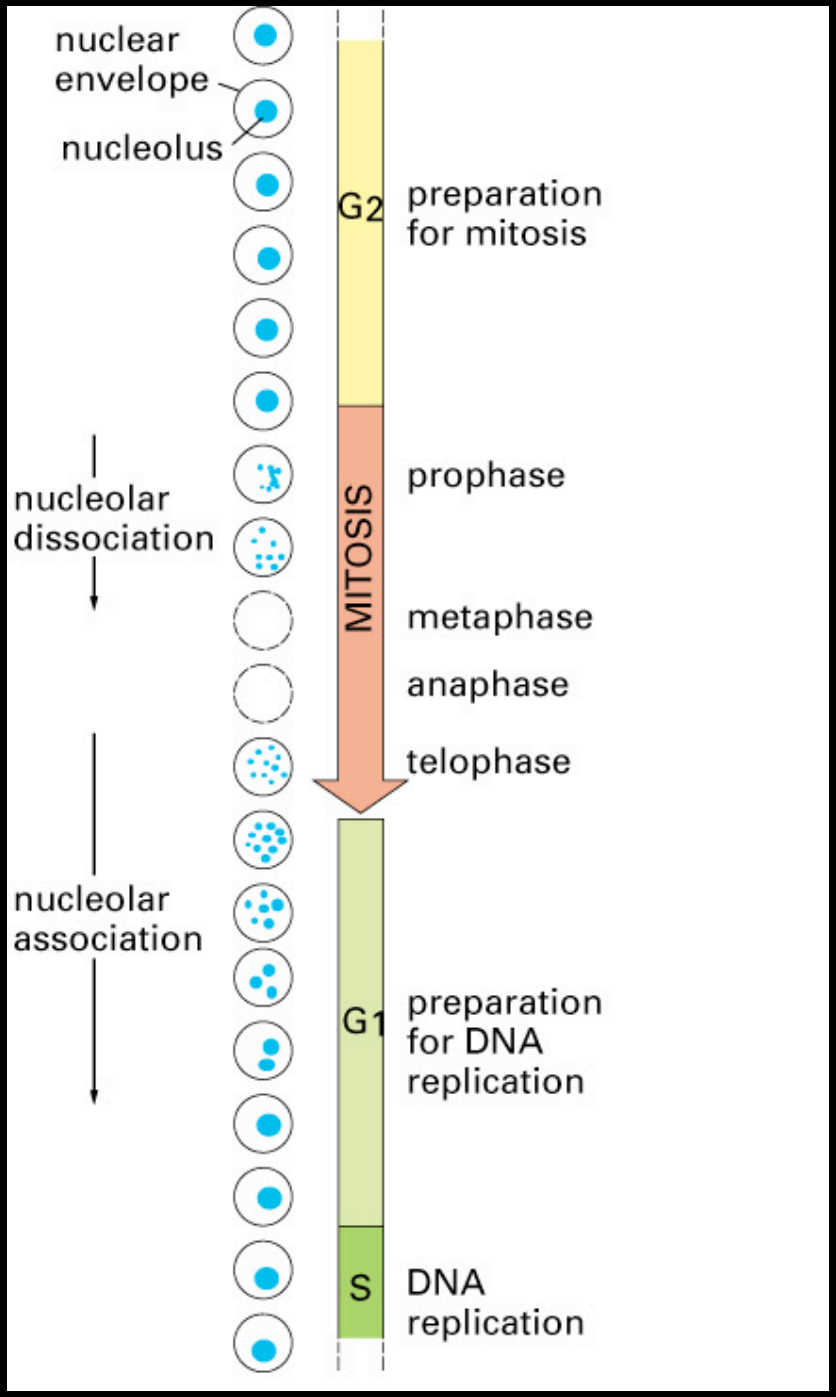
transcription and/or processing of mRNAs, snRNAs, histone mRNAs, and rRNA

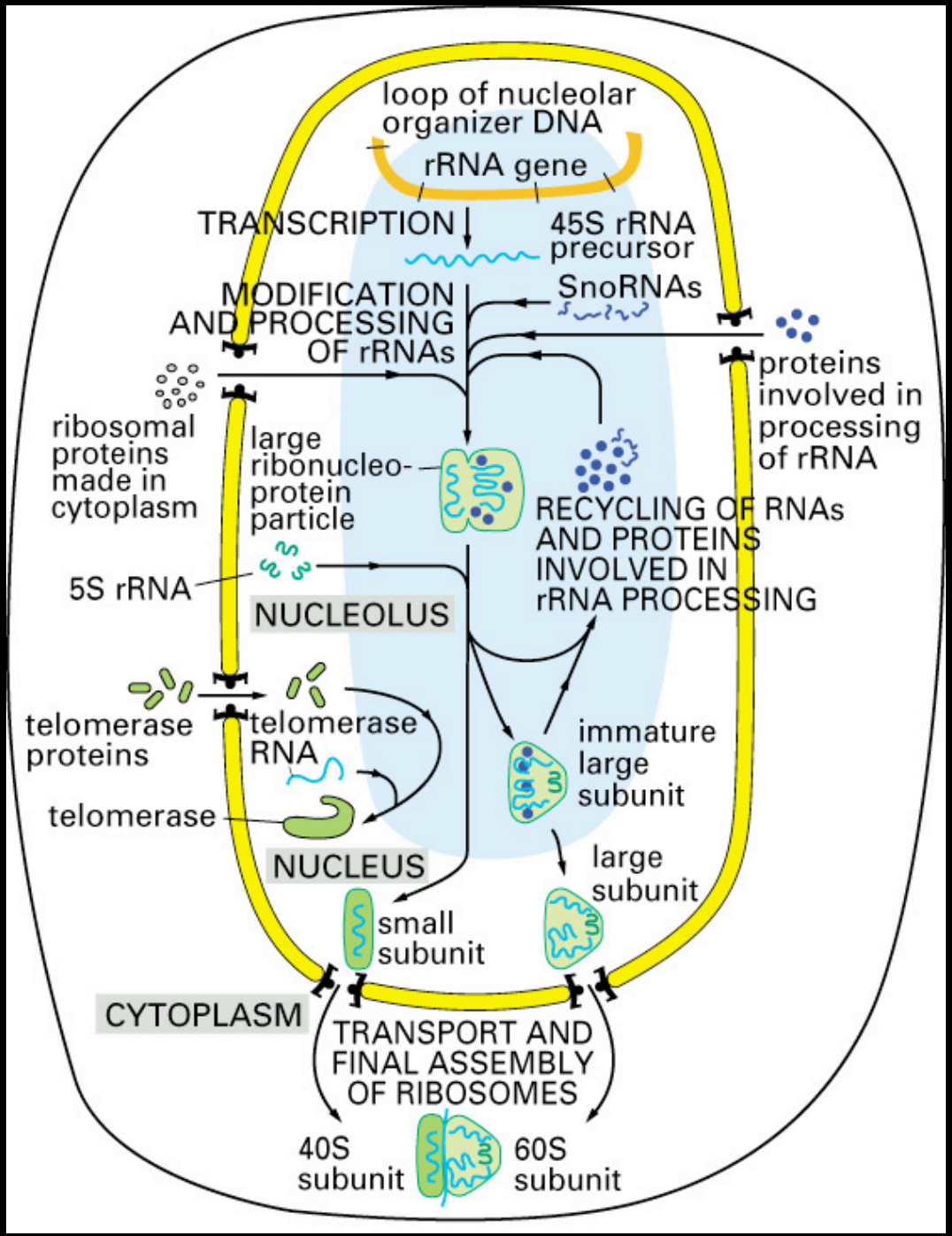
spatially associate with several U snRNA and histone gene loci (unusual 3' processing)

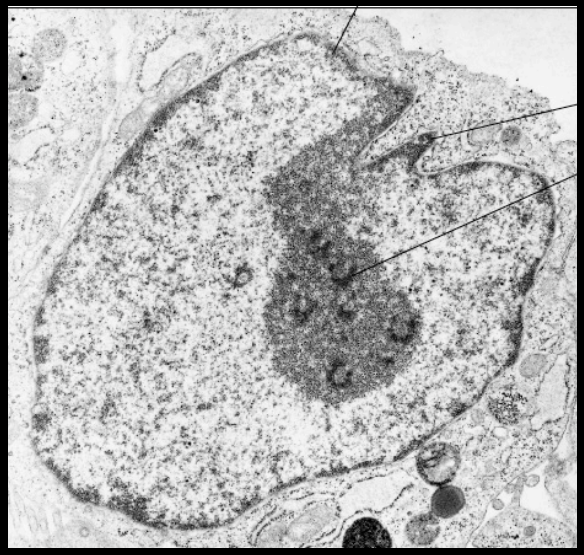
relationship to expression and nuclear metabolism of specific types of RNAs?

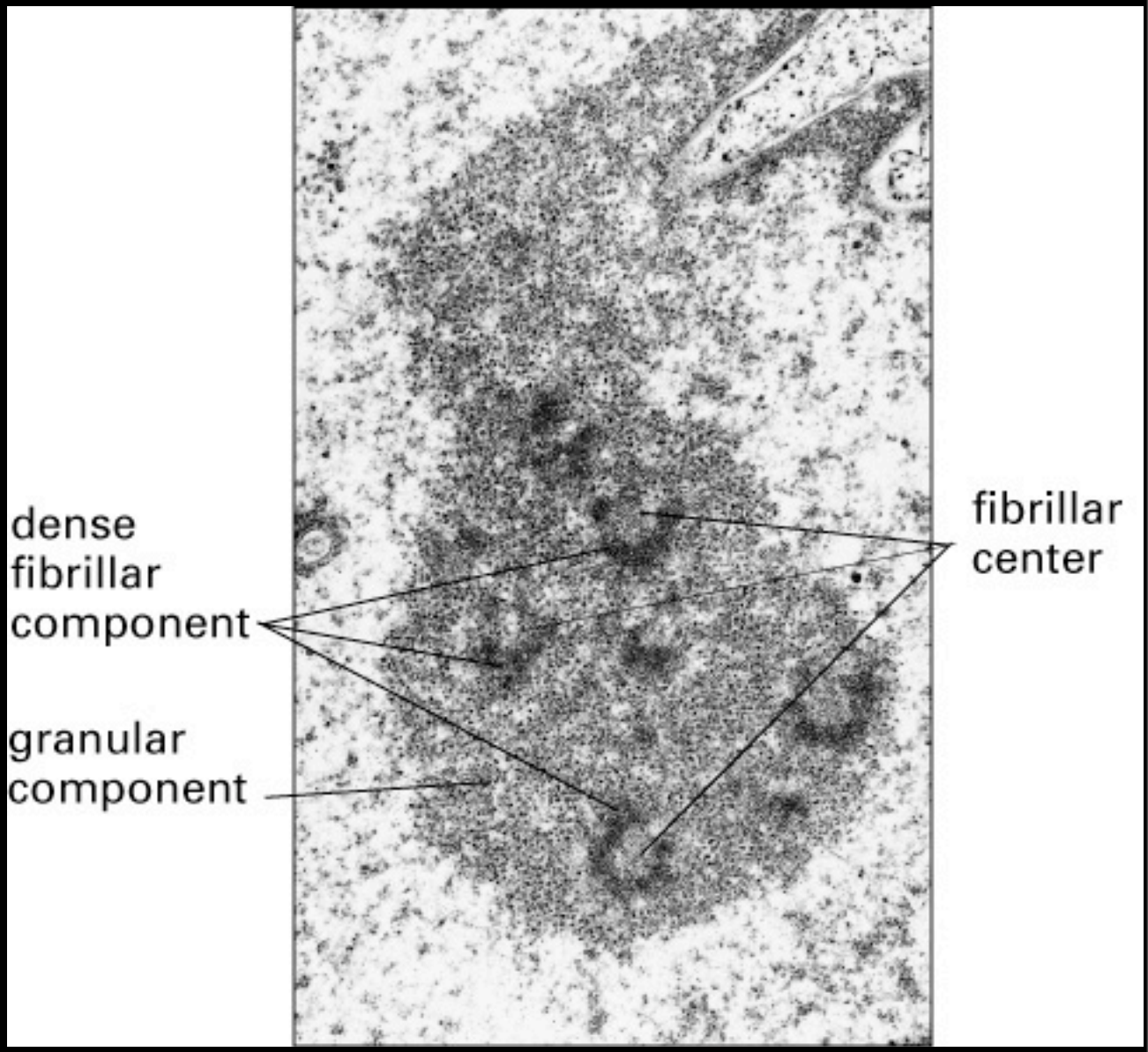
The Nucleolus: A Model for Formation of Nuclear Organelles



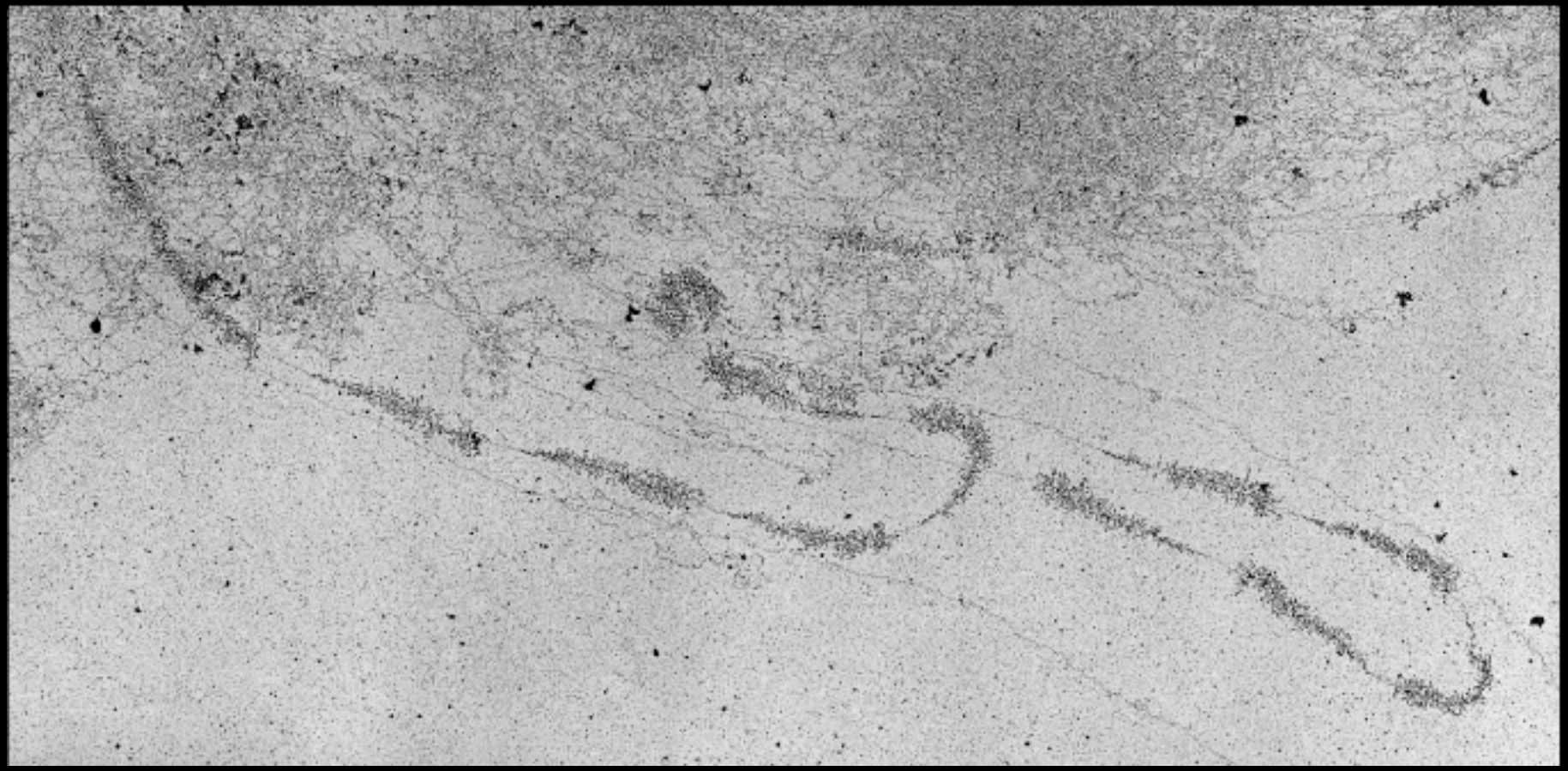



loop of nucleolar organizer DNA
rRNA gene
TRANSCRIPTION
45S rRNA precursor
SnoRNAs
MODIFICATION AND PROCESSING OF rRNAs
proteins involved in processing of rRNA
ribosomal proteins made in cytoplasm
large ribonucleo-protein particle
RECYCLING OF RNAs AND PROTEINS INVOLVED IN rRNA PROCESSING
5S rRNA
NUCLEOLUS
telomerase proteins
telomerase RNA
telomerase
immature large subunit
NUCLEUS
large subunit
small subunit
CYTOPLASM
TRANSPORT AND FINAL ASSEMBLY OF RIBOSOMES
40S subunit
60S subunit



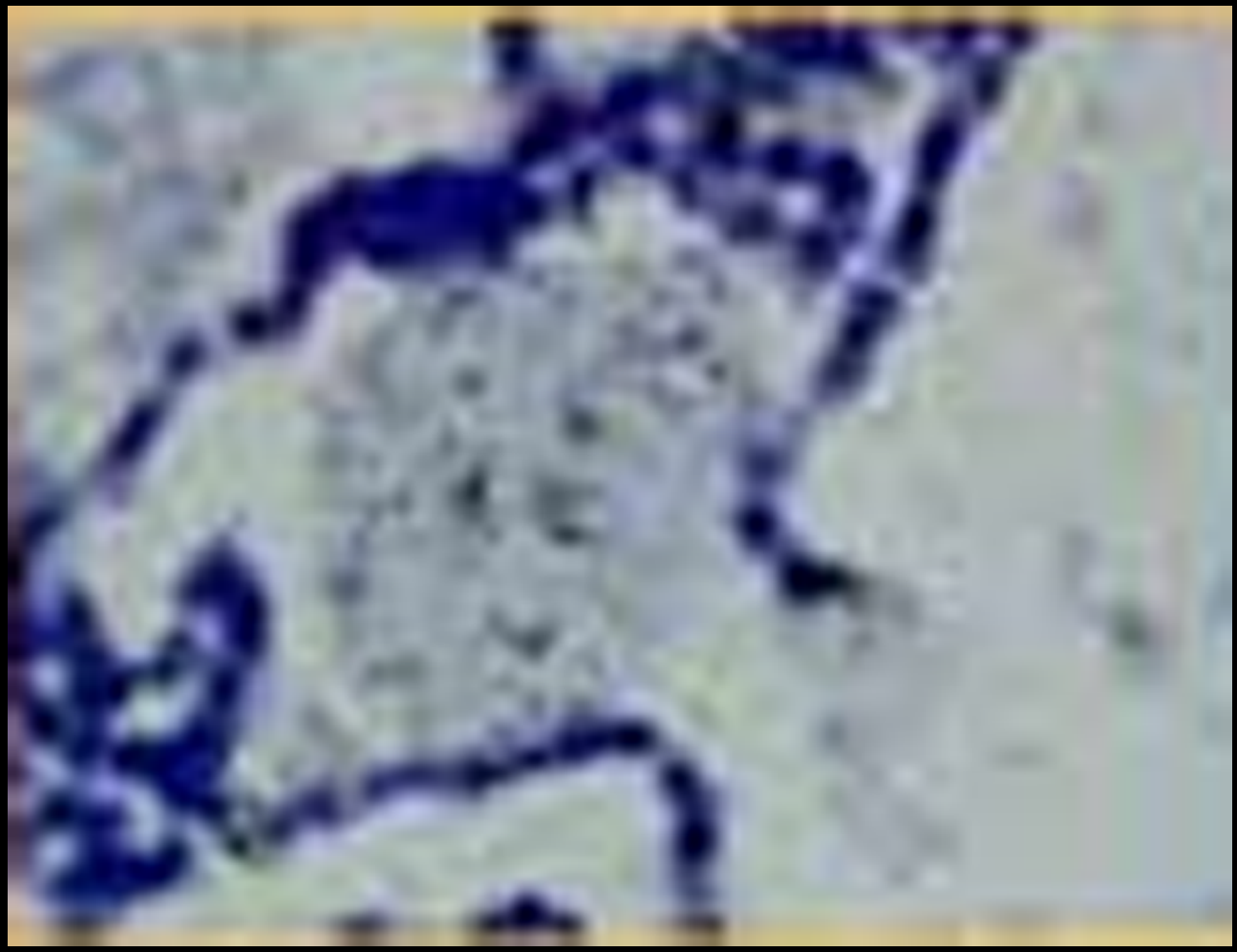

dense
fibrillar
component
granular
component
fibrillar
center

rDNA genes are the most highly transcribed genes in the genome



despite being embedded in heterochromatin

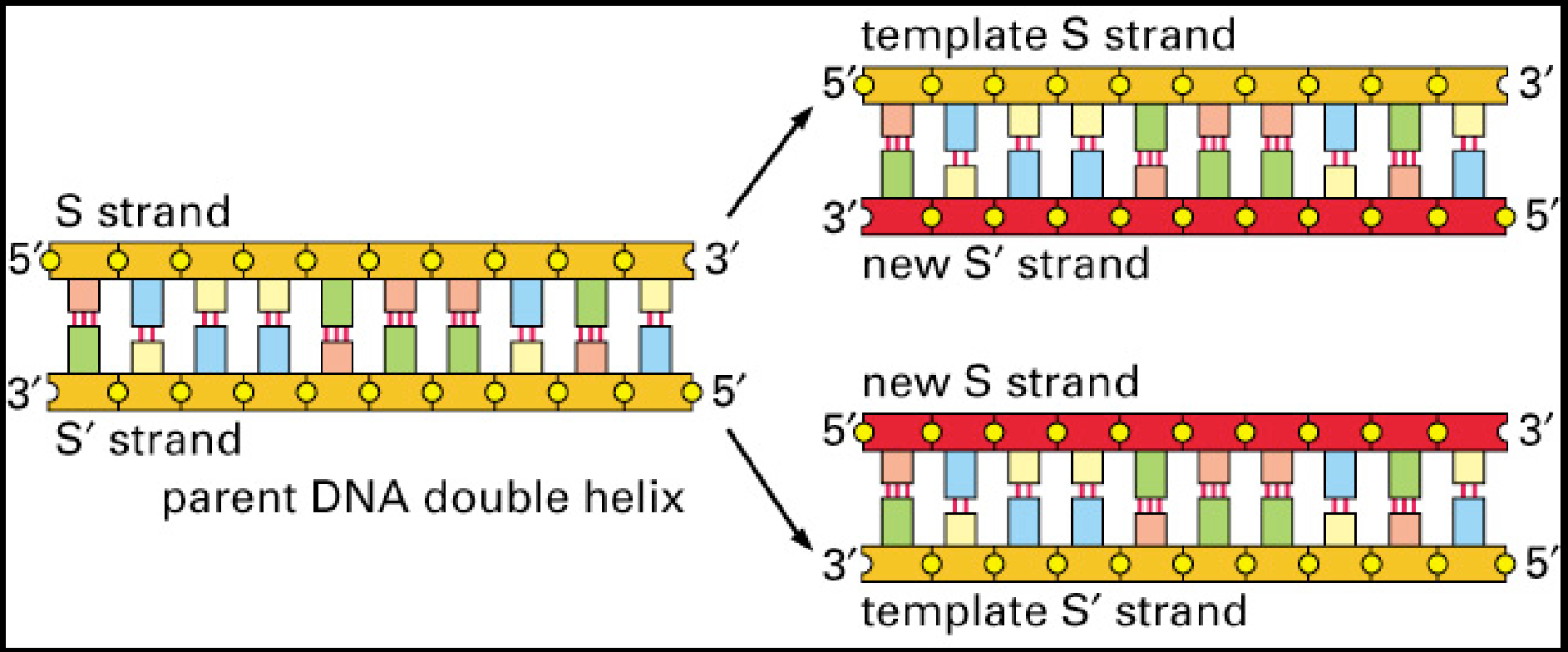
Mininucleoli form at ectopic sites of rDNA integration



Model for formation of a nuclear organelle:
self assembly around nucleating element
eg transcribed rRNA recruits nucleolar components

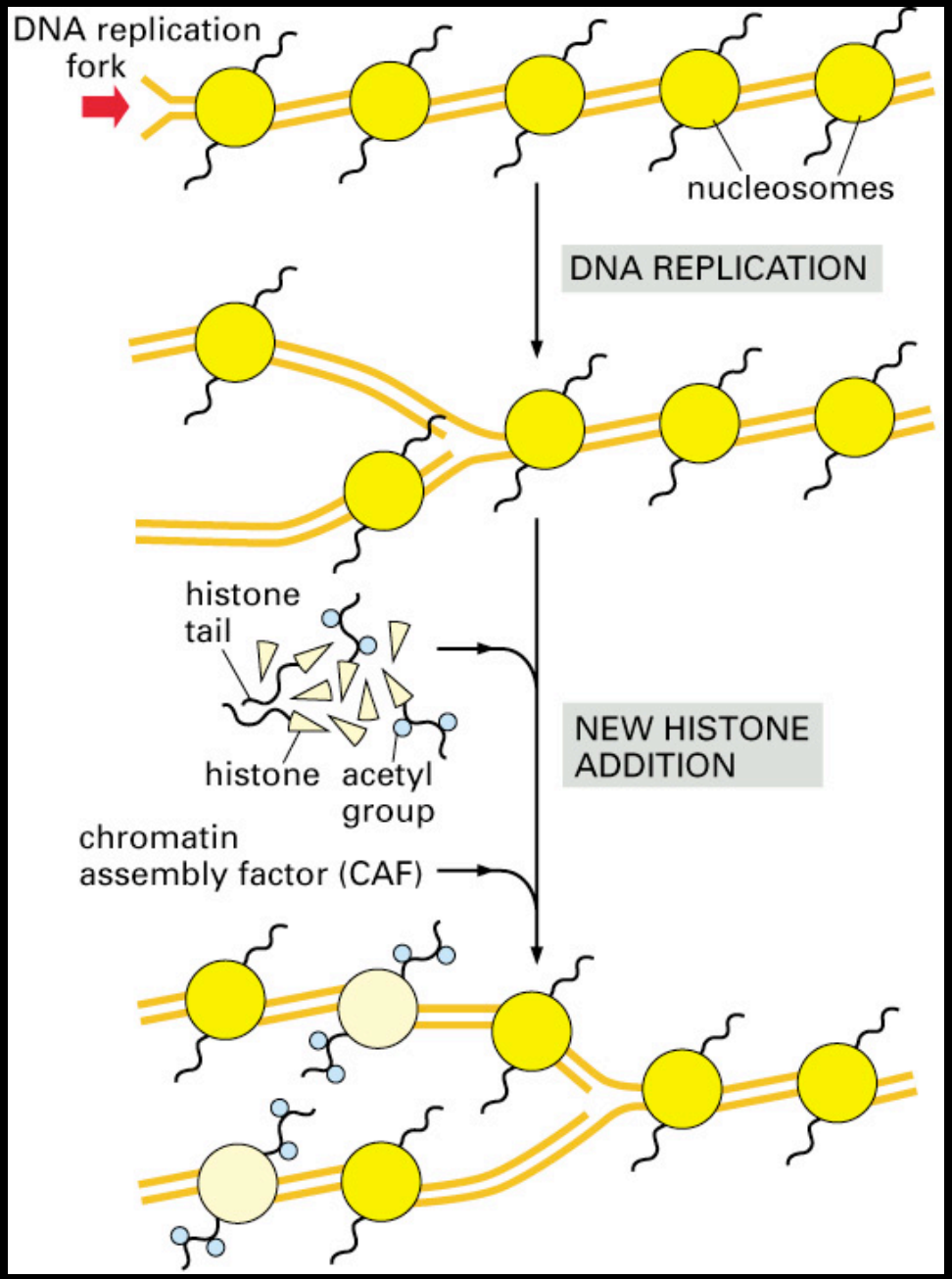
Chromatin Structure and DNA Replication

DNA replication is ‘simple’....

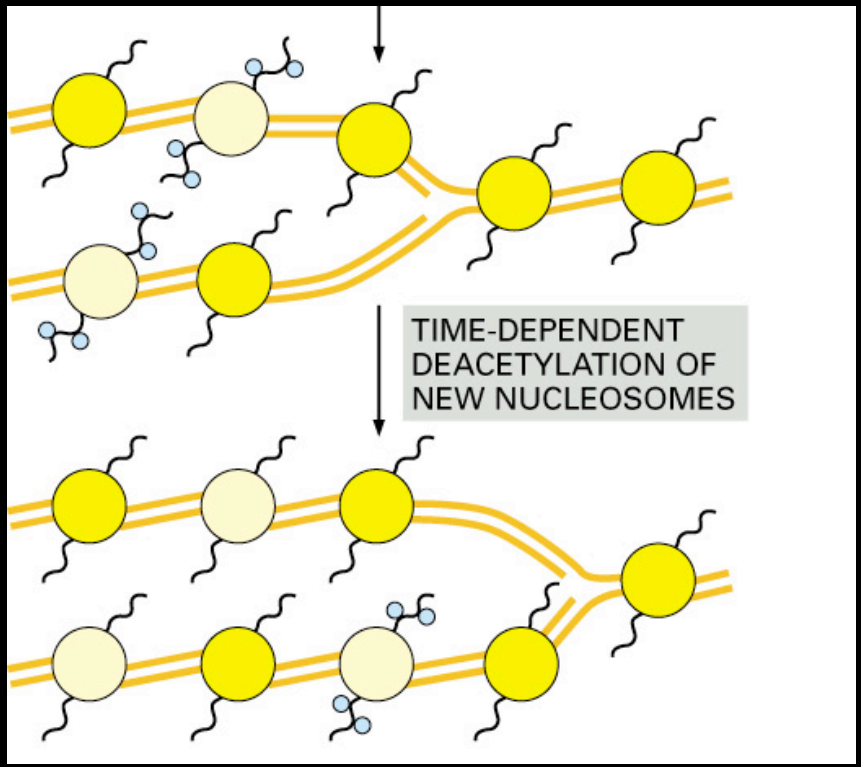


...though the regulation of molecules (e.g. DNA polymerases, helicases) is not...

Chromatin is also ‘Replicated’



Epigenetic Patterns are Reestablished after Replication

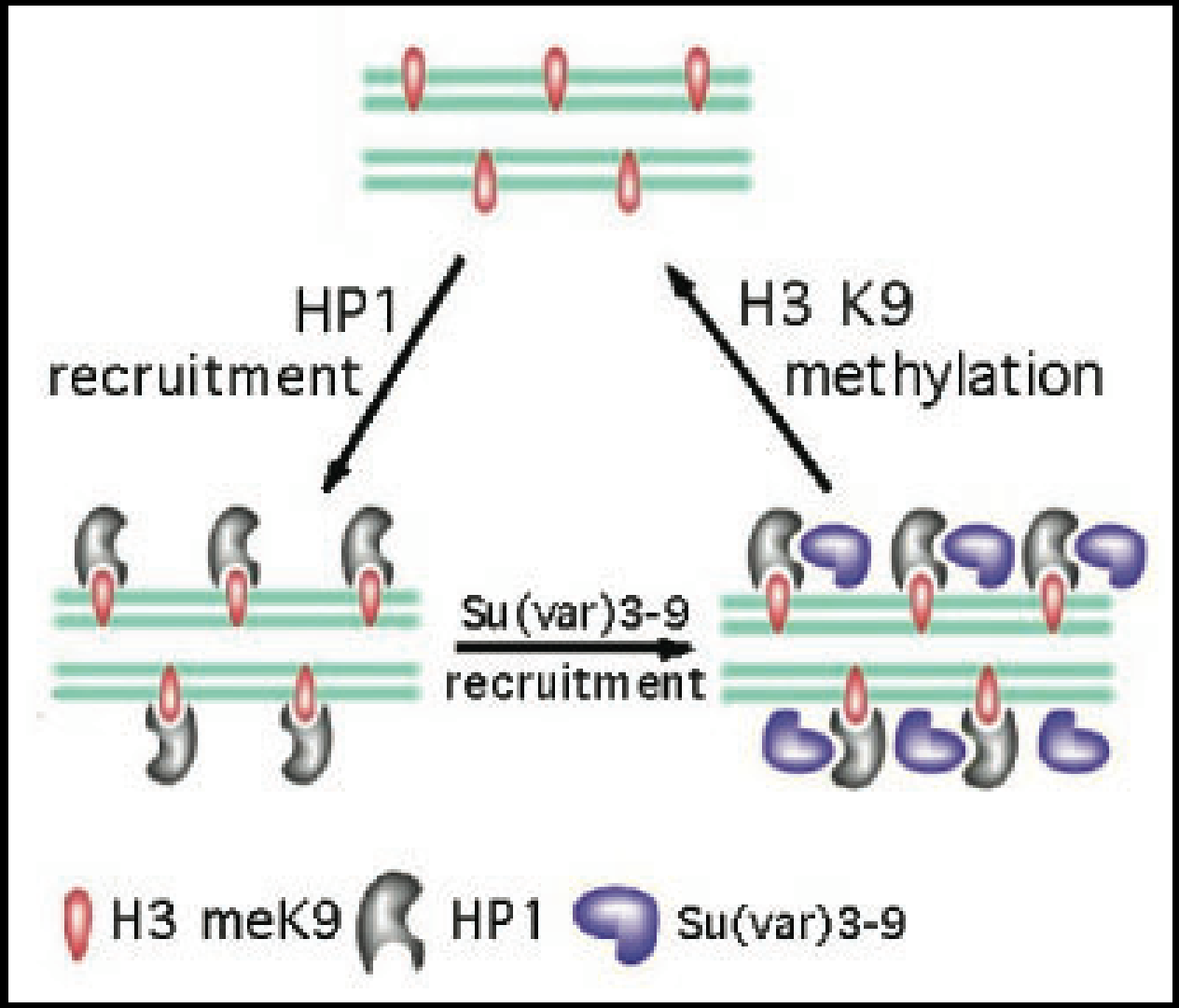


propagation of modifications in parental nucleosomes

Propagation of a ‘Silent’ Epigenetic State

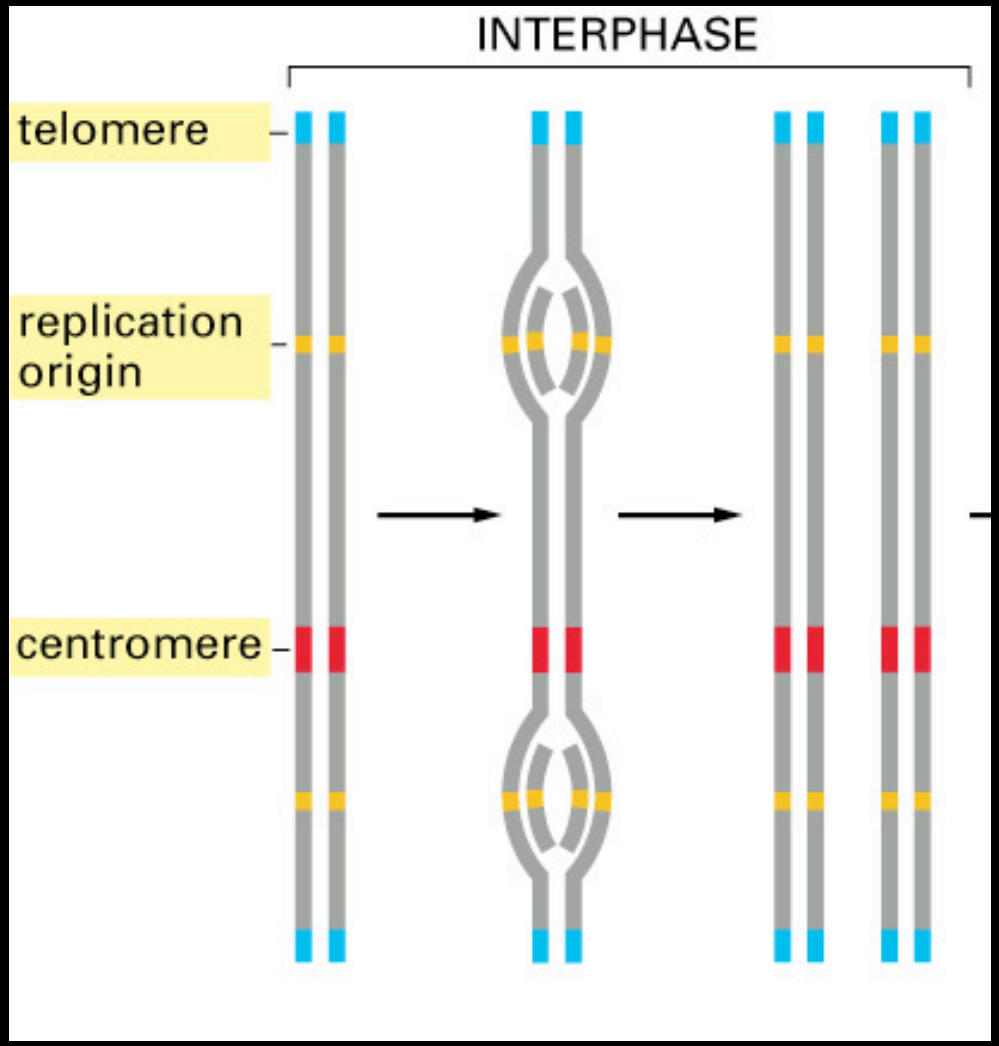
Su(var)3-9 : H3 K9 methyltransferase

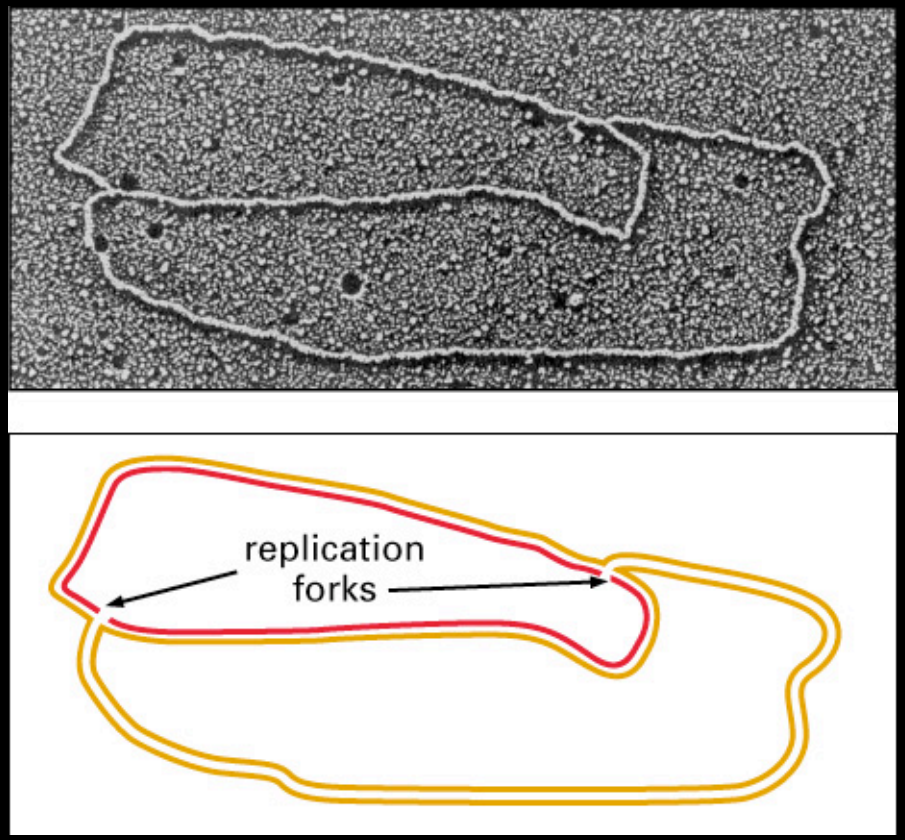
HP1 / Su(var)2-5 : chromodomain, binds H3 K9 Me & 3-9



Jenuwein

Replication is Initiated at ‘Origins’

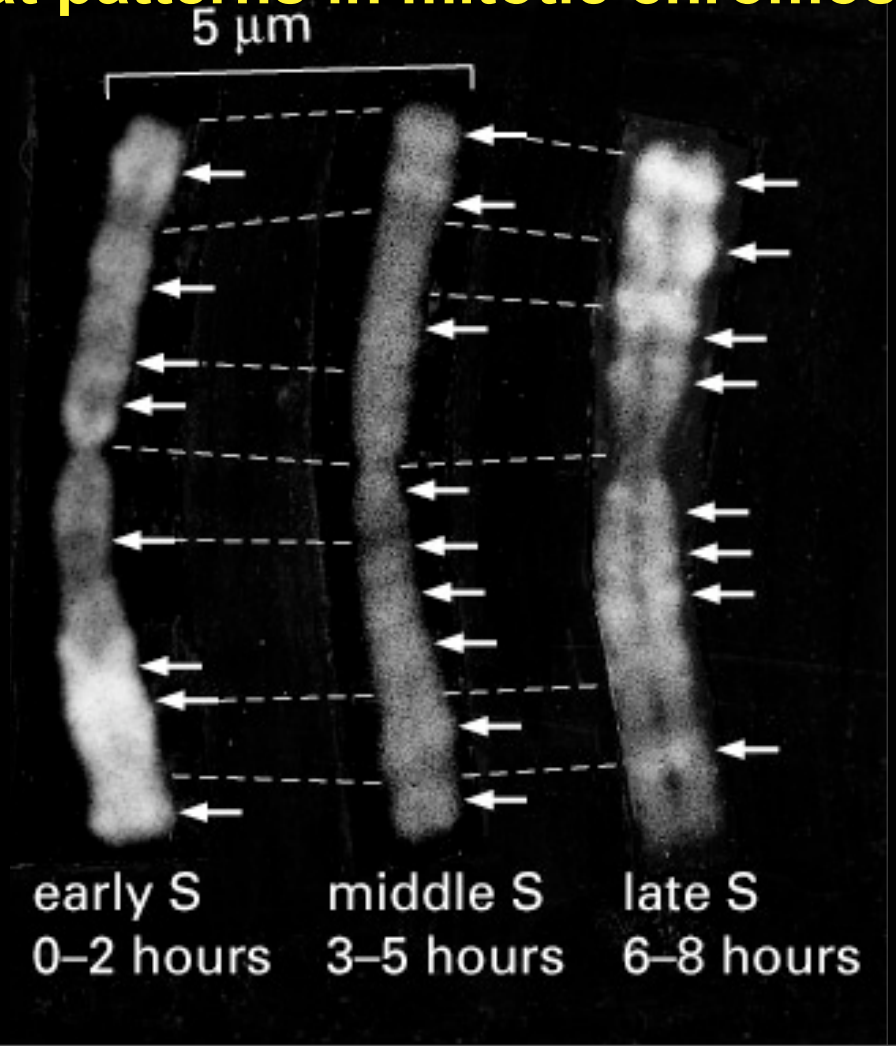




normally sequence dependent in cerevisiae, probably epigenetic in higher euks

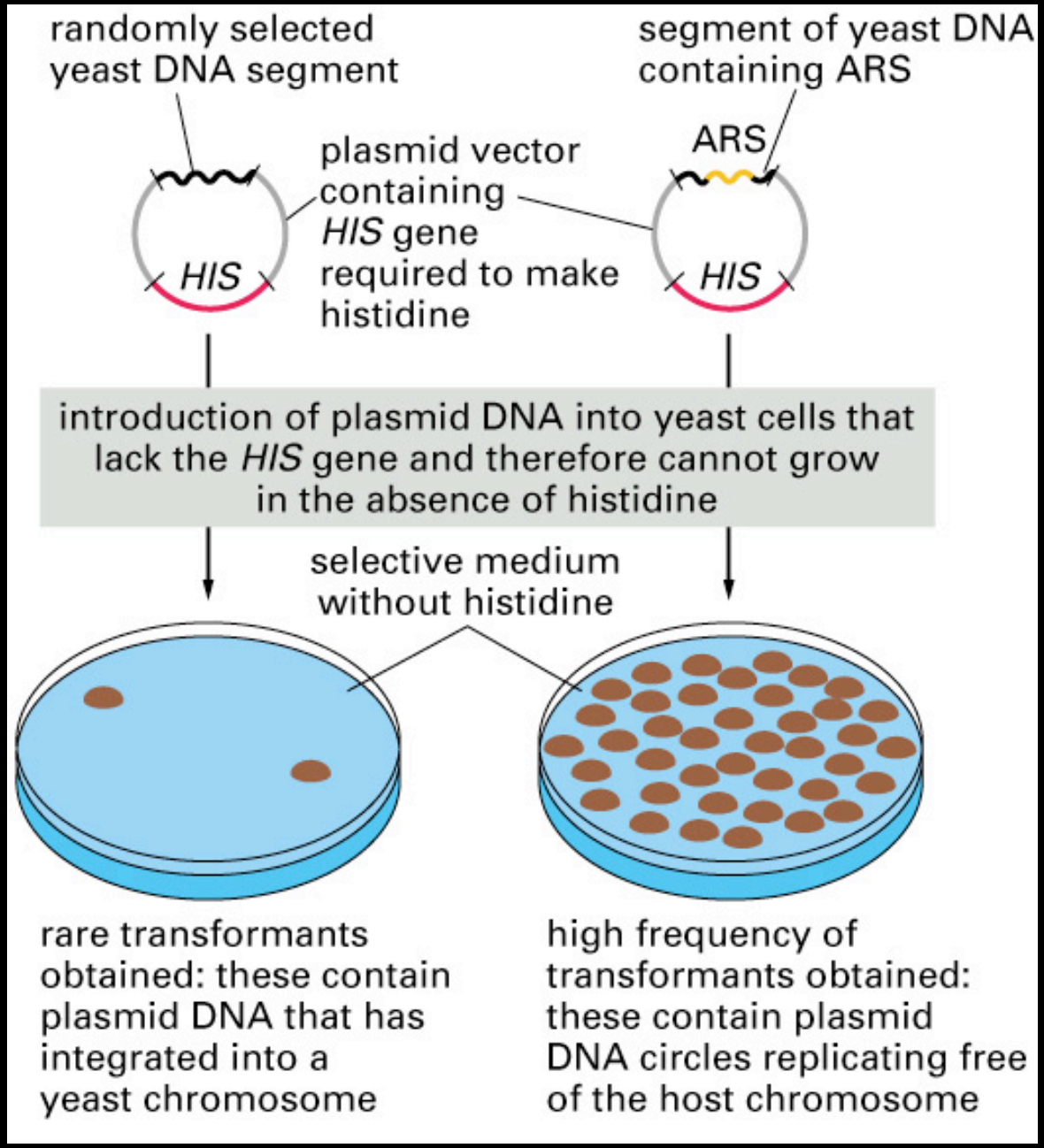
Temporal Control of Replication during S phase

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

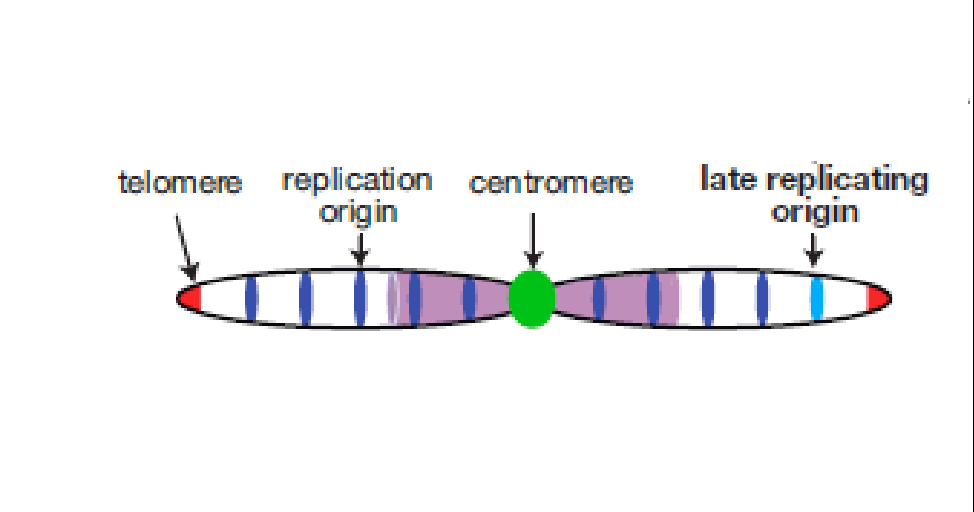
identification of ARSs (Autonomously Replicating Sequences)



Replication Timing and Activity is Chromatin-Dependent in Yeast

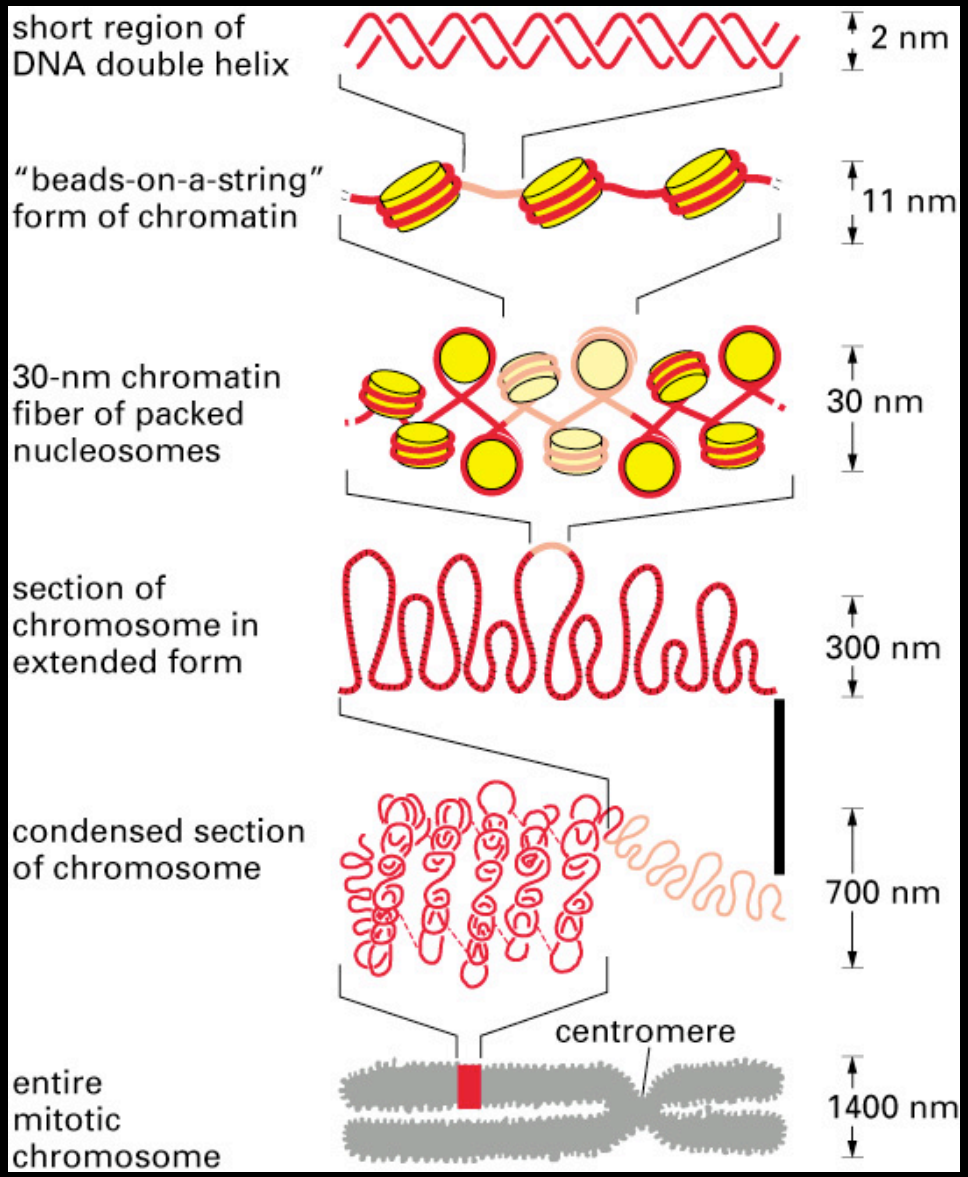
each rDNA copy (in tandem arrays) contain an ARS, but only 20% ‘fire’

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

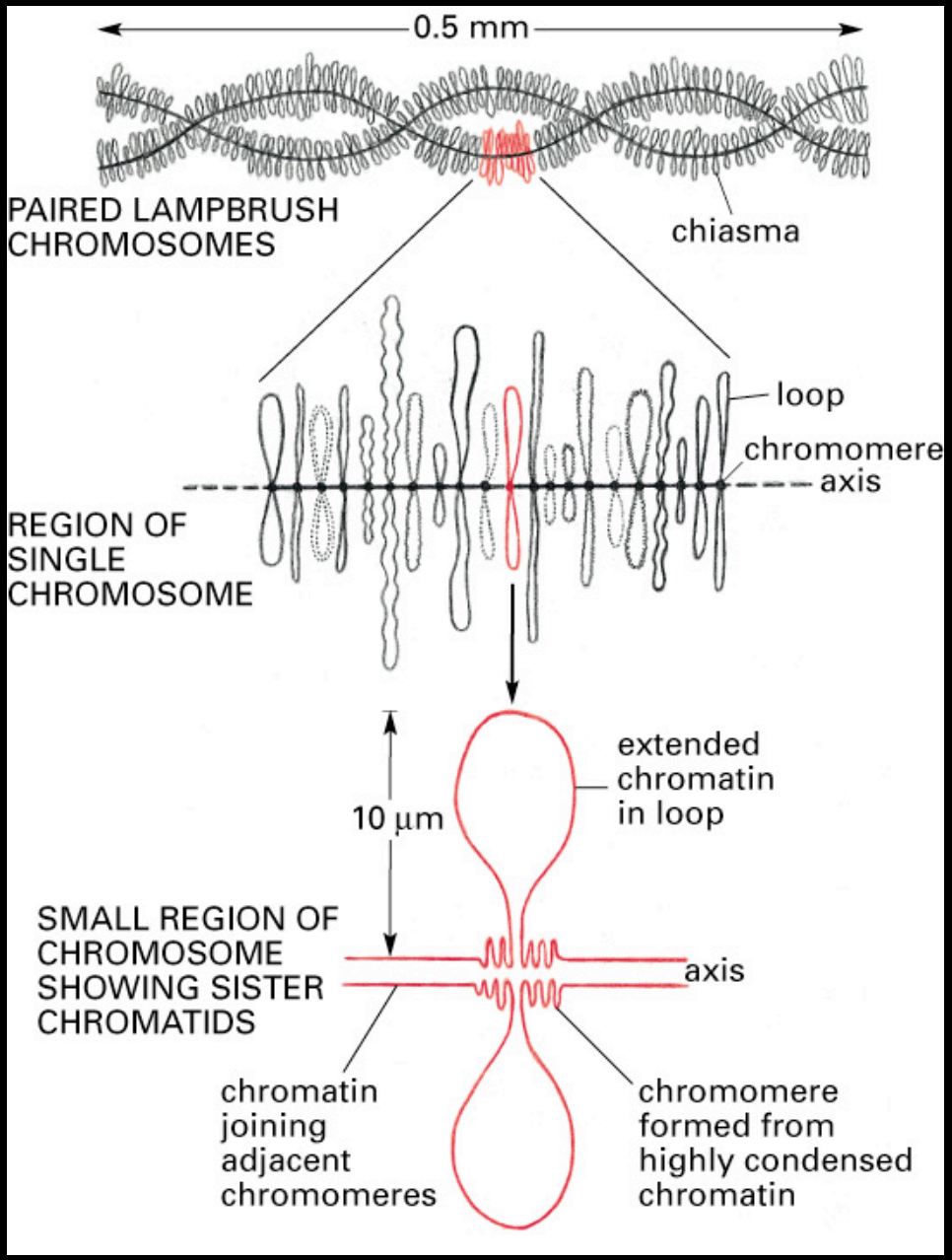


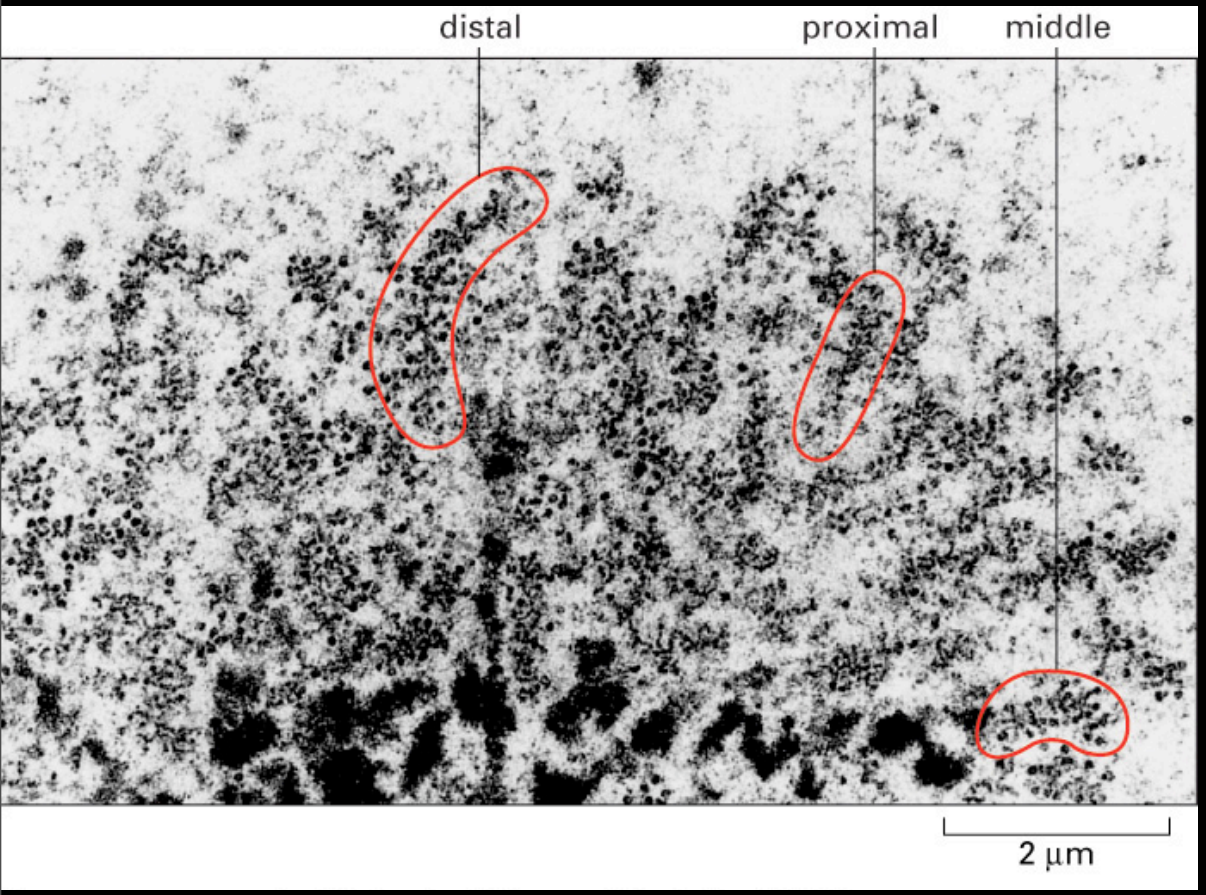
(Ferguson and Fangman 1992; Weinreich et al. 2004)

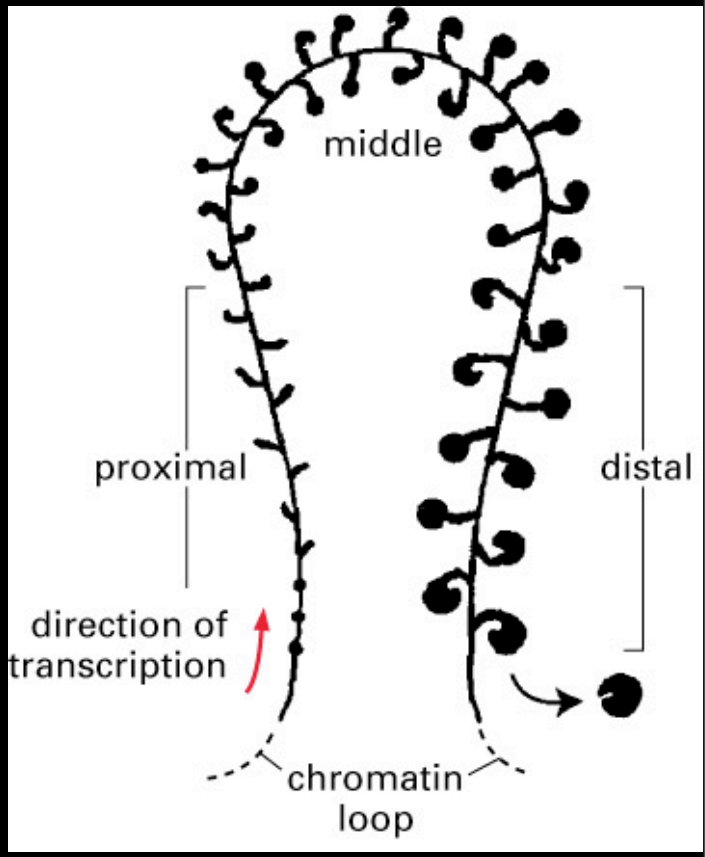
Gene expression is also dependent on chromosome structure and nuclear organization

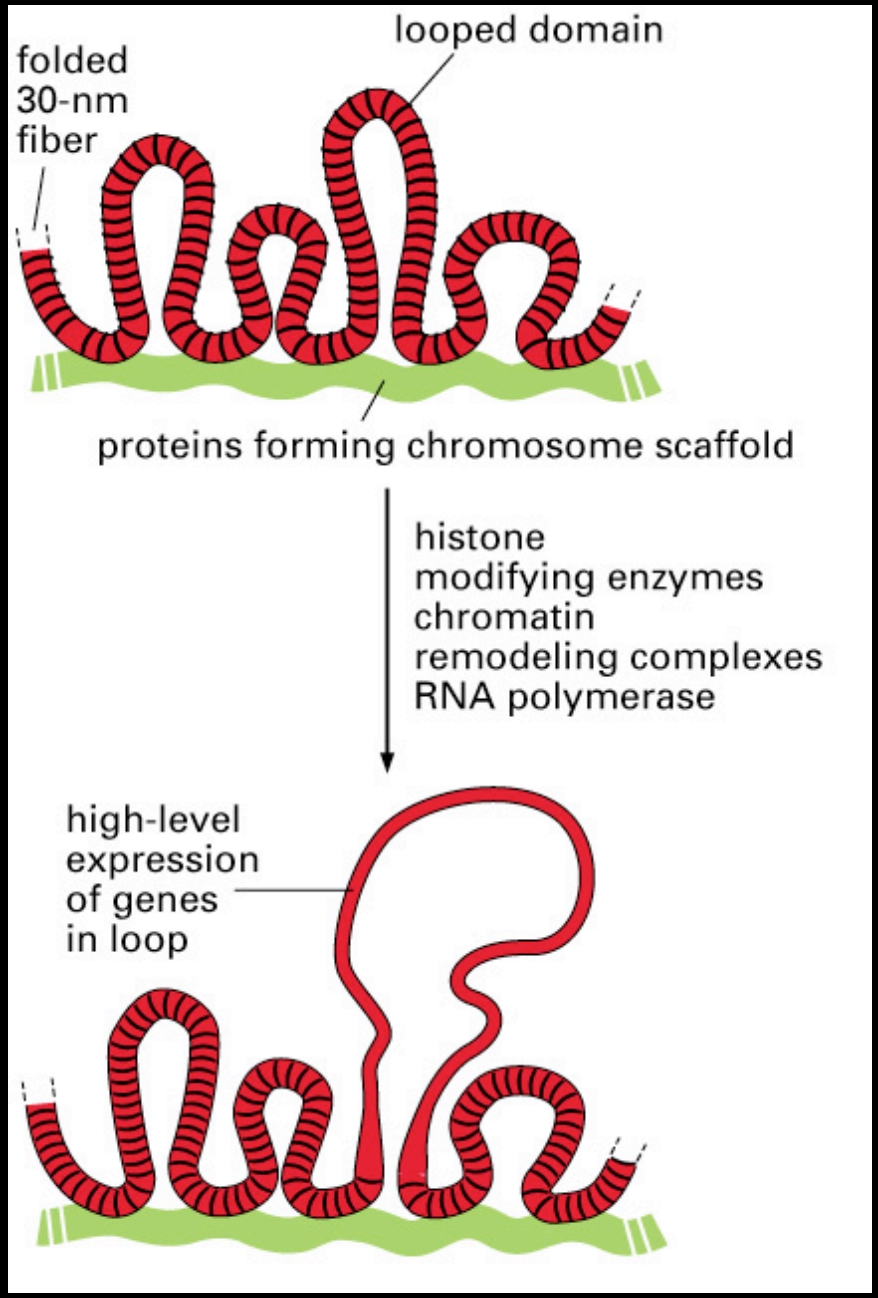


Lampbrush loops in Amphibian Oocytes

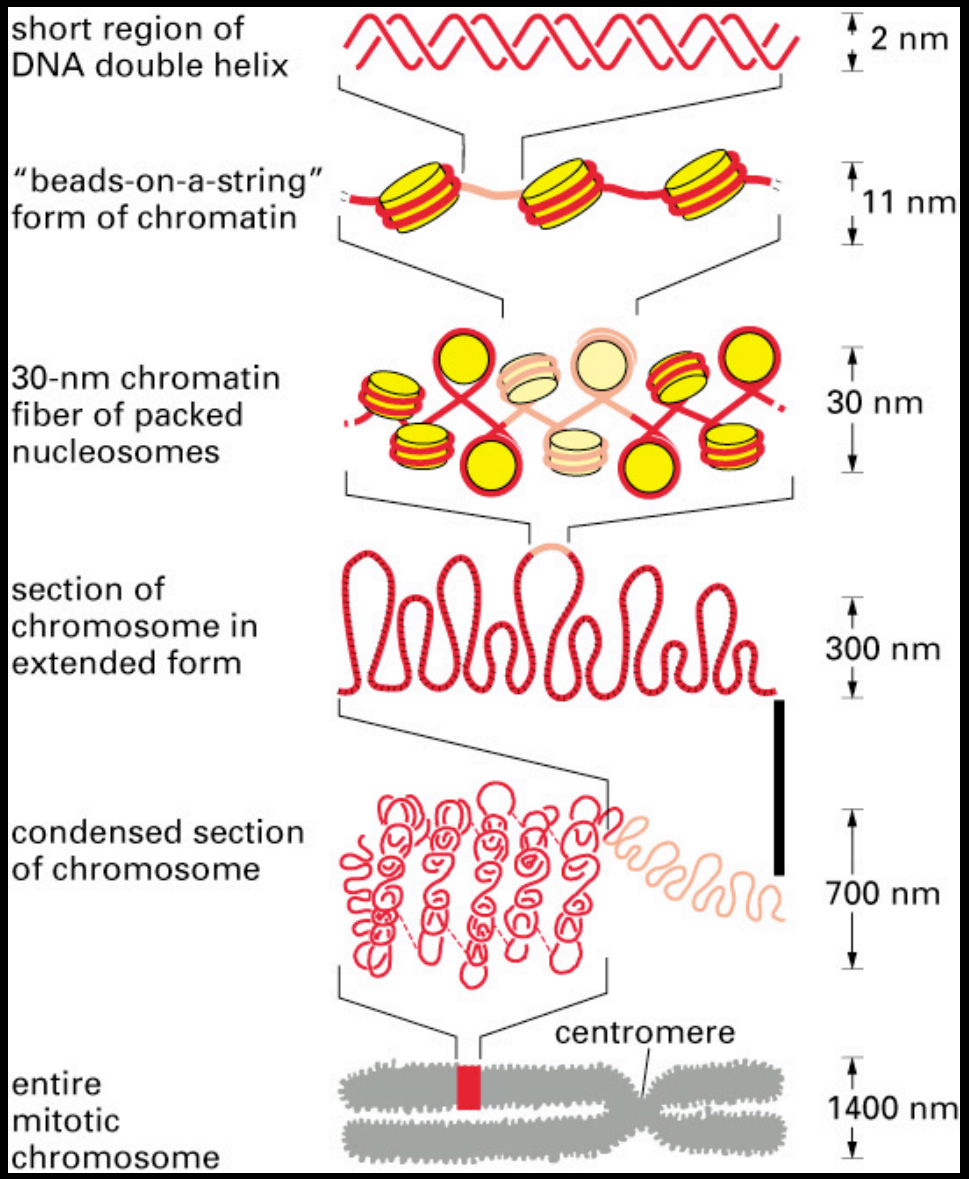


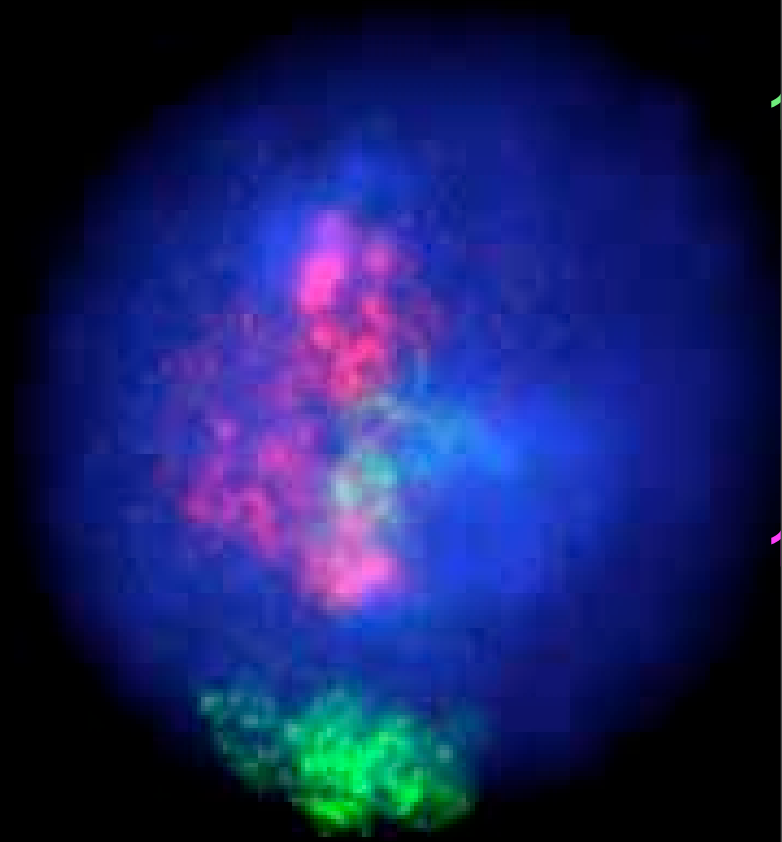

distal
proximal
middle
2 µm


middle
proximal
distal
direction of transcription
chromatin loop

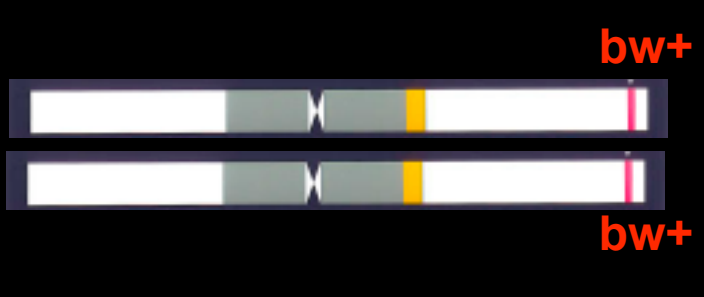

looped domain
folded
30-nm
fiber
proteins forming chromosome scaffold
histone
modifying enzymes
chromatin
remodeling complexes
RNA polymerase
high-level
expression
of genes
in loop

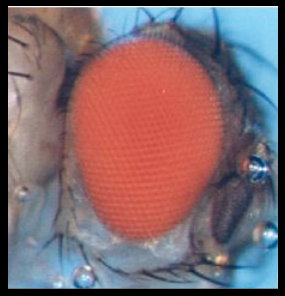
Effects on expression in the context of nuclear architecture



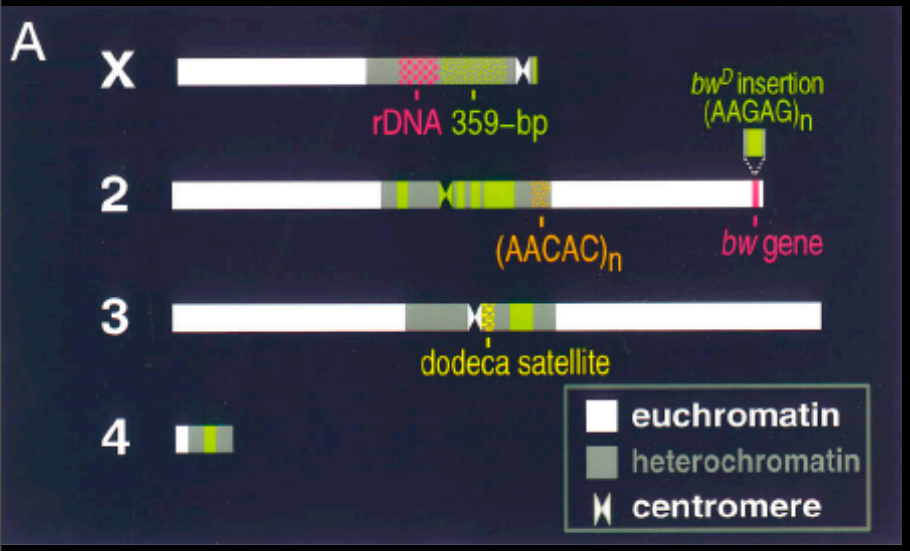


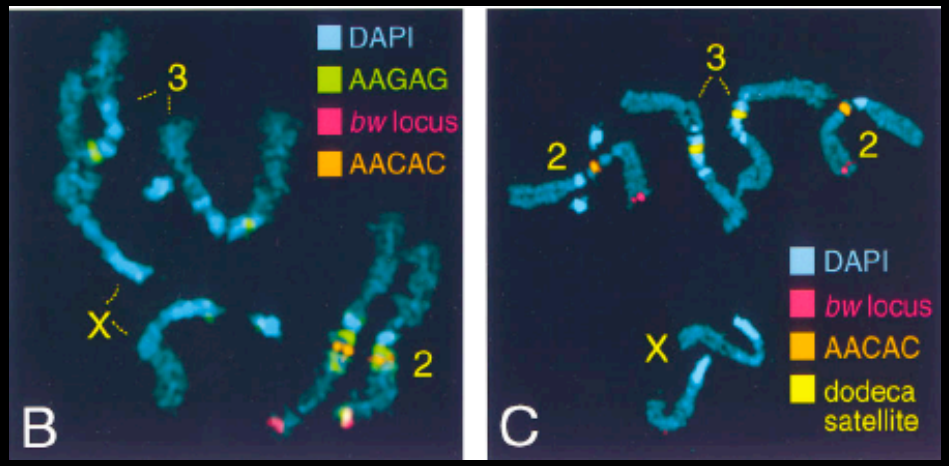
Brown-Dominant: A model for effects of nuclear organization of chromosomes on gene expression





bw expressed

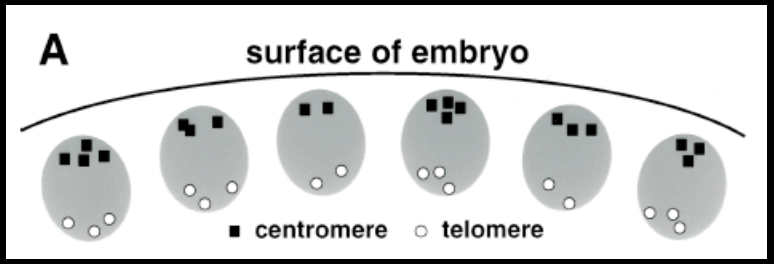


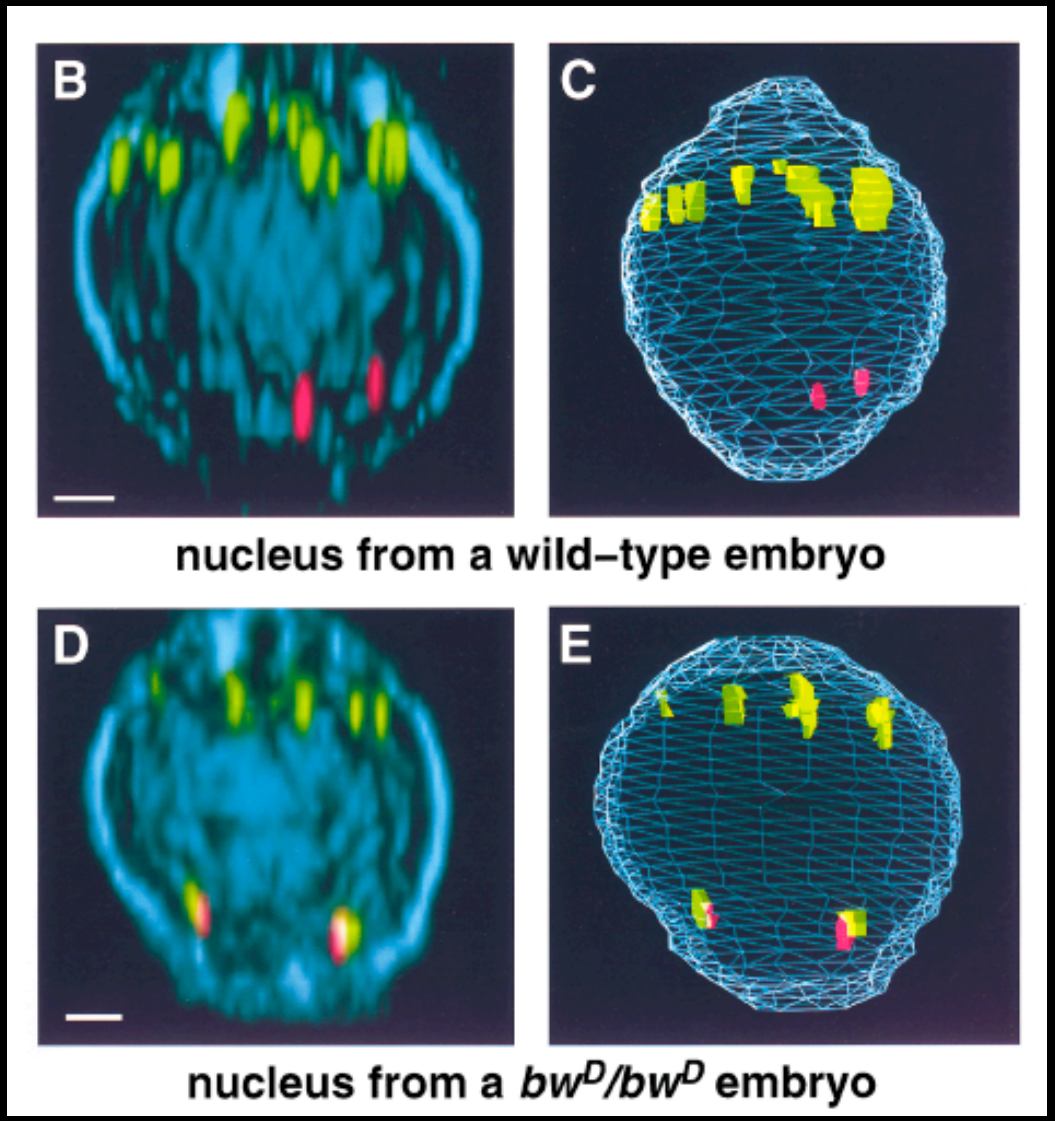


use FISH probes-mark positions of bw and heterochromatin (satellites)

bw

AAGAG



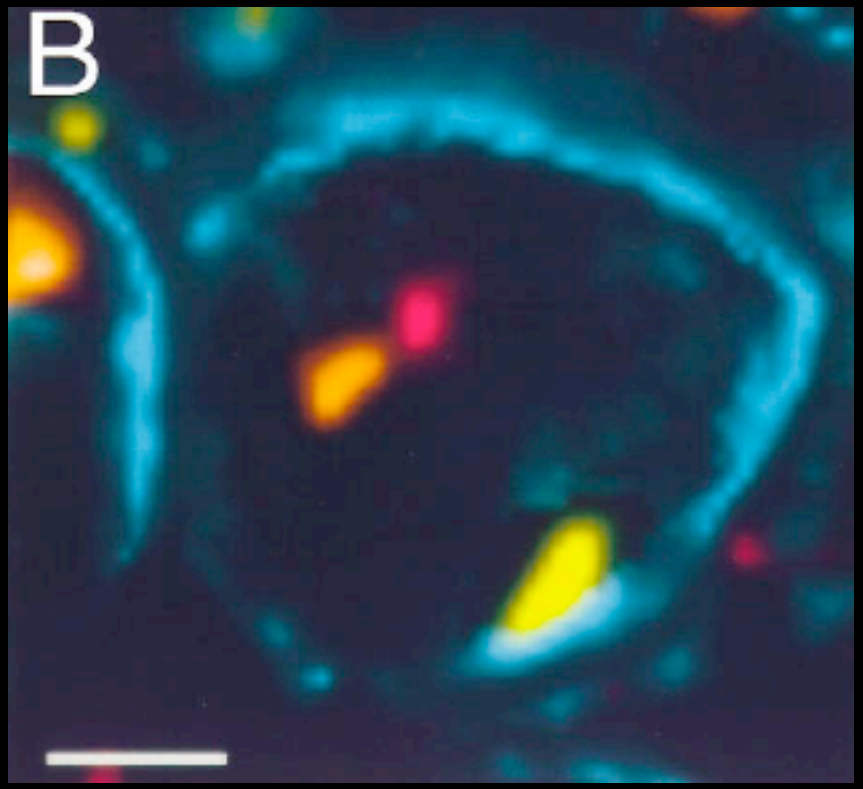


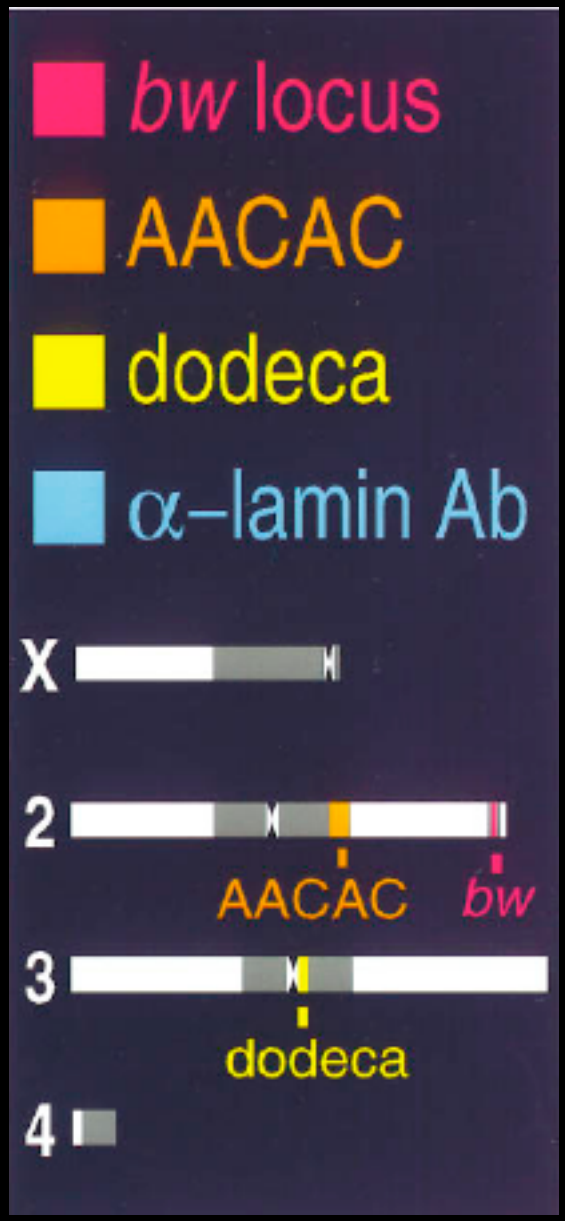
bw normally basal, AAGAG apical

identify position of bw-D by costaining

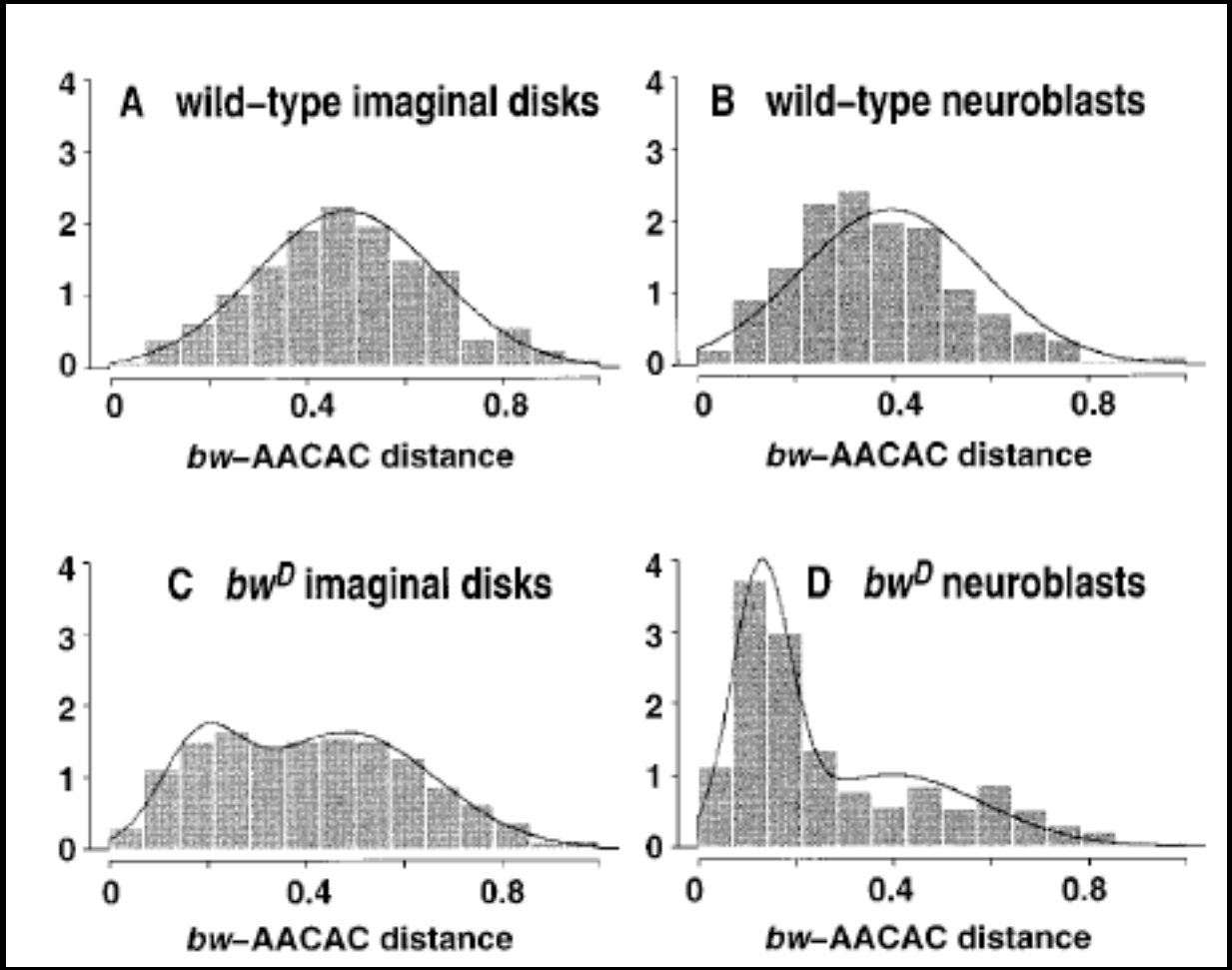
Brown-Dominant associates with heterochromatin in tissues relevant to eye expression

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+

Thursday

Mitotic chromosome structure and function