Chromosome Morphology & Number

Karyotype describes the chromosome complement of an individual or species in terms of number, size, and morphology of its chromosomes

Idiogram is a diagrammatic representation of the chromosomes, based on observations and measurements in a number of cells

Karyotypes can be based on mitotic or meiotic chromosomes and are enhanced by chromosome banding techniques. The largest pair of chromosomes is usually designated as number 1 and the rest are numbered in order of their relative diminishing size, to the smallest pair.

Chromosome landmarks

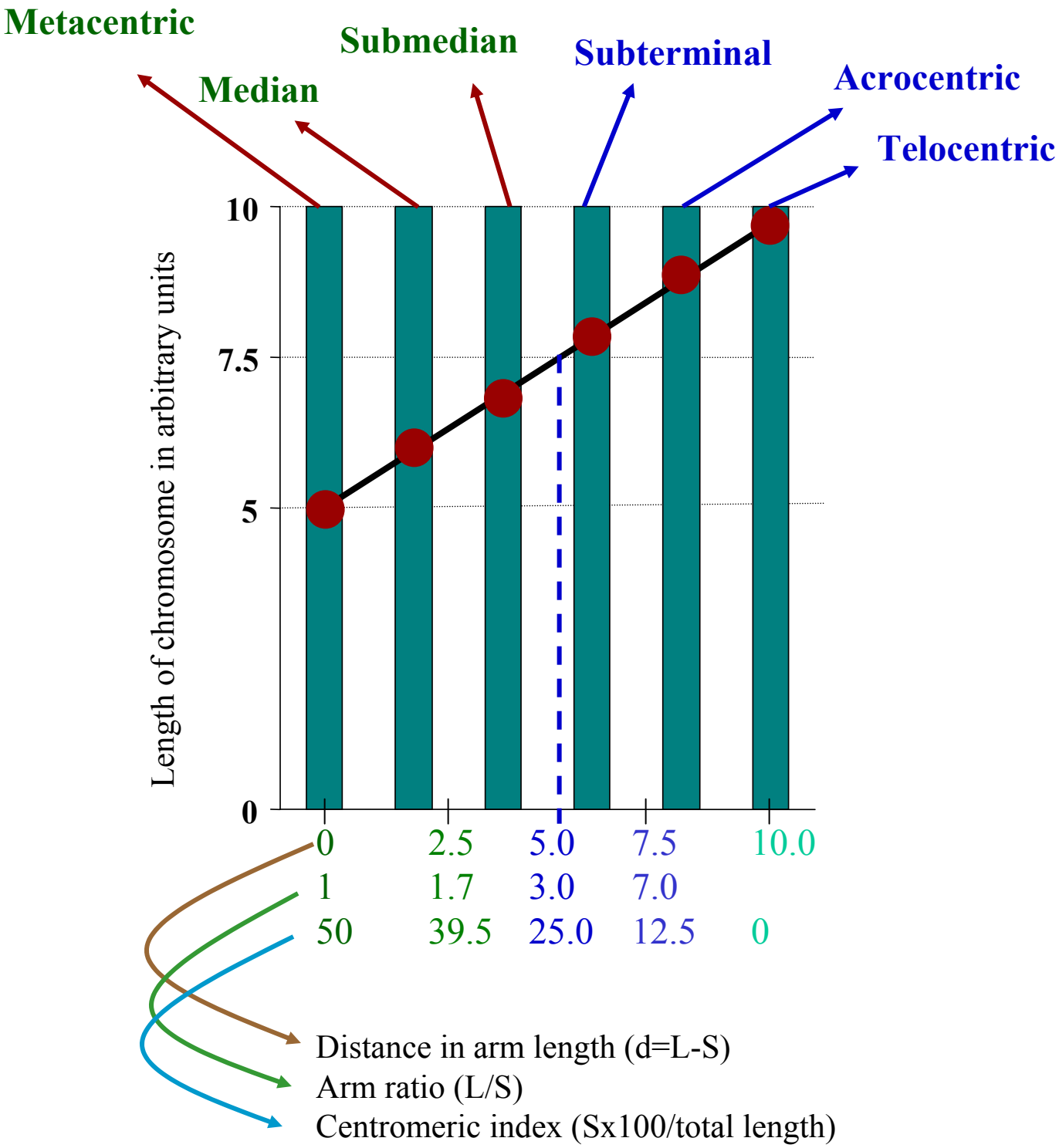
Primary constriction	The position of centromere defines chromosome arms (telocentric, acrocentric, subterminal, submedian, median, metacentric)
Secondary constriction	Satellites which define the locations of ribosomal RNA genes
Tertiary constriction	Only in some species and often correspond to the sites of cold-sensitive hetrochromatic regions

Mitotic karyotype analysis

Number of chromosomes
Morphology of chromosomes
Heterochromatin vs. Euchromatin
Chromosome Banding
Labeling (i.e. *in situ* hybridization)

Meiotic karyotype analysis

Number of chromosomes
Morphology of chromosomes (more detailed than mitotic analysis)
Heterochromatin vs. Euchromatin (more detailed than mitotic analysis)
Chromosome Banding
Labeling (i.e. *in situ* hybridization)
Chromosome pairing behavior
Gene expression (in certain situations)



L= Long arm; S=Short arm

Chemicals used to observe chromosomes

Category	Chemical
Stimulation of cell division	Phytohemagglutinin stimulates lymphocytes to divide
Cell synchronization	Hydroxyurea; Amethopterin (folic acid analog); Cold treatment
Spindle dispersion and spreading of chromosomes	Colchicine, α-bromonaphthalene; 8-hydroxyquinoline; hypotonic treatment (20-30% isotonic)
Staining chromosomes	Feulgen (DNA-specific staining); Giemsa, Carmine

Chromosome Banding Techniques

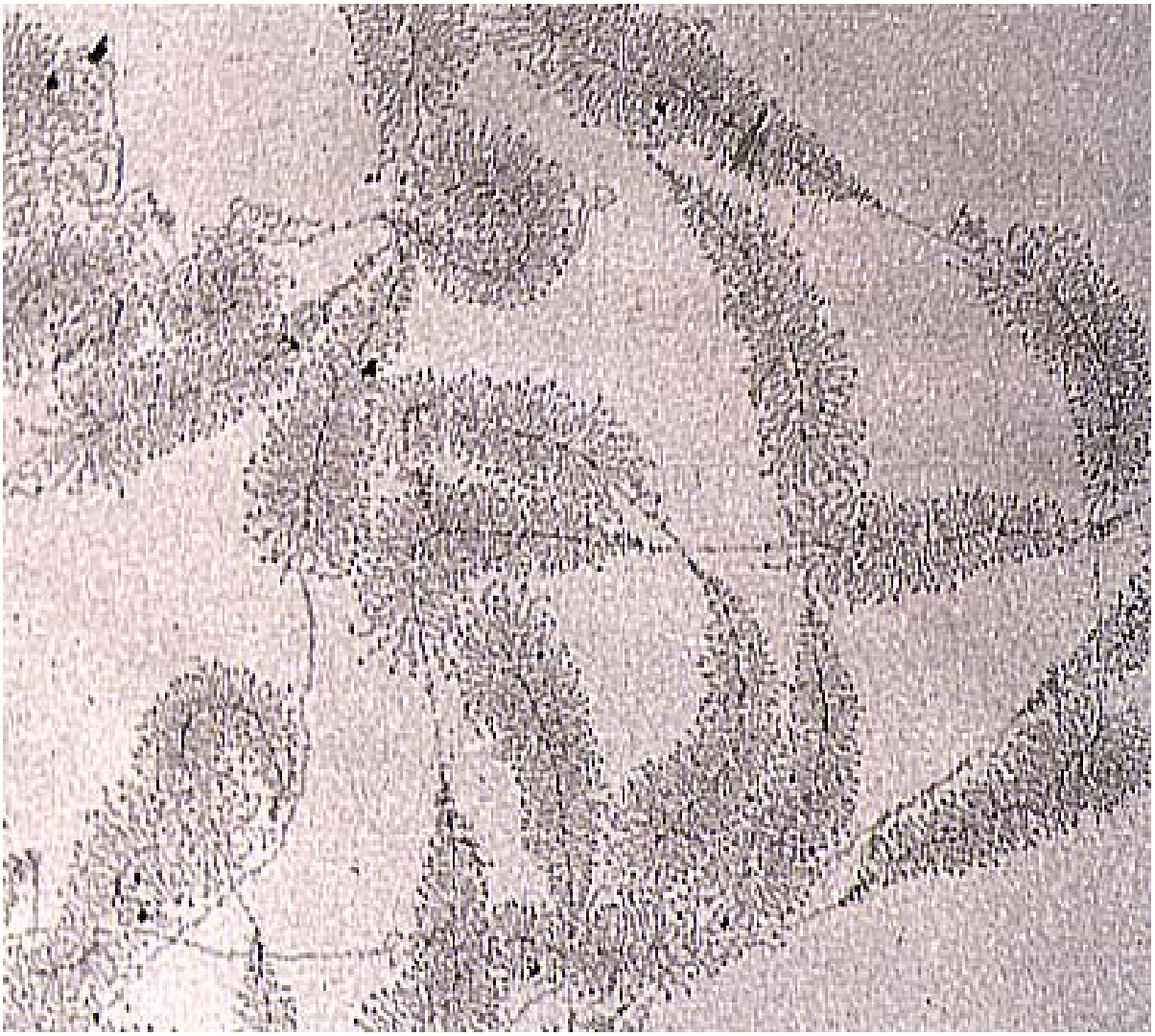
Name	Basis	Organisms Studied
Q-banding	Banding of AT-specific fluorochromes to DNA	Reptiles, birds, mammals
G-banding	Giemsa staining after incubation in warm SSC or trypsin	Fish, amphibia, reptiles, birds, mammals
R-banding	Giemsa staining after incubation in hot buffer	Mammals
C-banding	Giemsa staining after alkali treatment	Most plants and animals
Replication banding	Incorporation of BrdU during either early or late S phase followed by Giemsa staining	Plants, vertebrates

Chromosome Banding Techniques

C-Banding	Giemsa stains the satellite-rich centromeric heterochromatin. Therefore C-bands reveal the location of **constitutive heterochromatin**
R-Banding	R bands apparently contain **GC rich DNA** and are the **interbands in G-banded** chromosomes.
G-Banding	Dark bands (G-bands) alternating with light bands are produced in prophase or metaphase chromosomes. The G-bands are region of the chromosome where the DNA is presumably free to stack with the dyes in the stain.
Q-Banding	**AT rich DNA** reacts strongly with quinacrine and enhances its fluorescence in UV while GC rich DNA quenches the fluorescence.

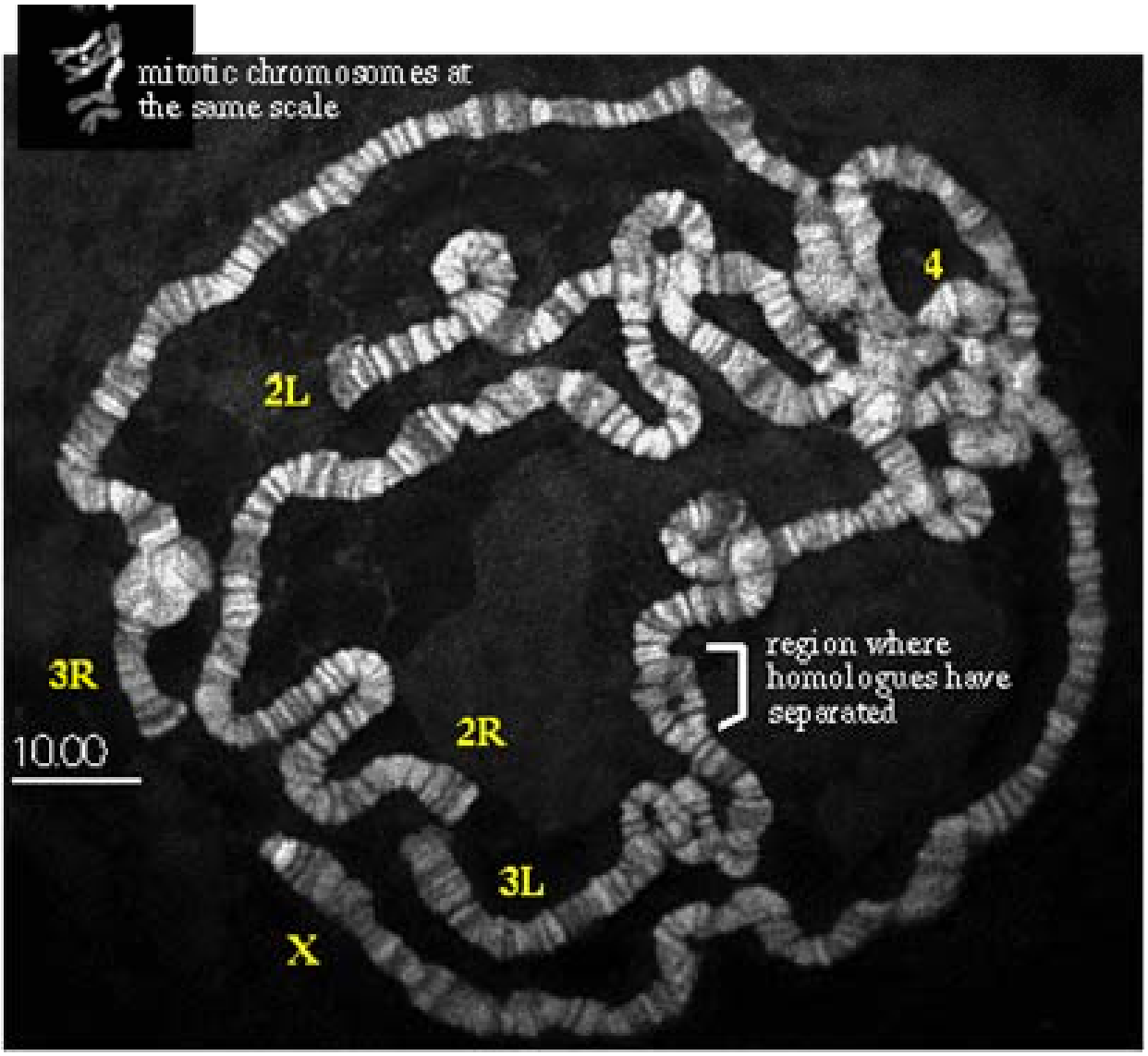
Lampbrush chromsomes

In the arrested diplotene (dictyoene) chromosomes expand and transcription begins. In oocytes of certain vertebrates this transcription is evident under light microscopy.



Polytene chromosomes

DNA replication takes place but mitosis is arrested at the G_2 stage. The replicated chromatids of each chromosome remain attached to one centromere. This process of endoreduplicaton can lead to a final levels of 1024 DNA strands in the polytene chromosomes of *Dorsophila melanogaster* salivary glands.



Telomere

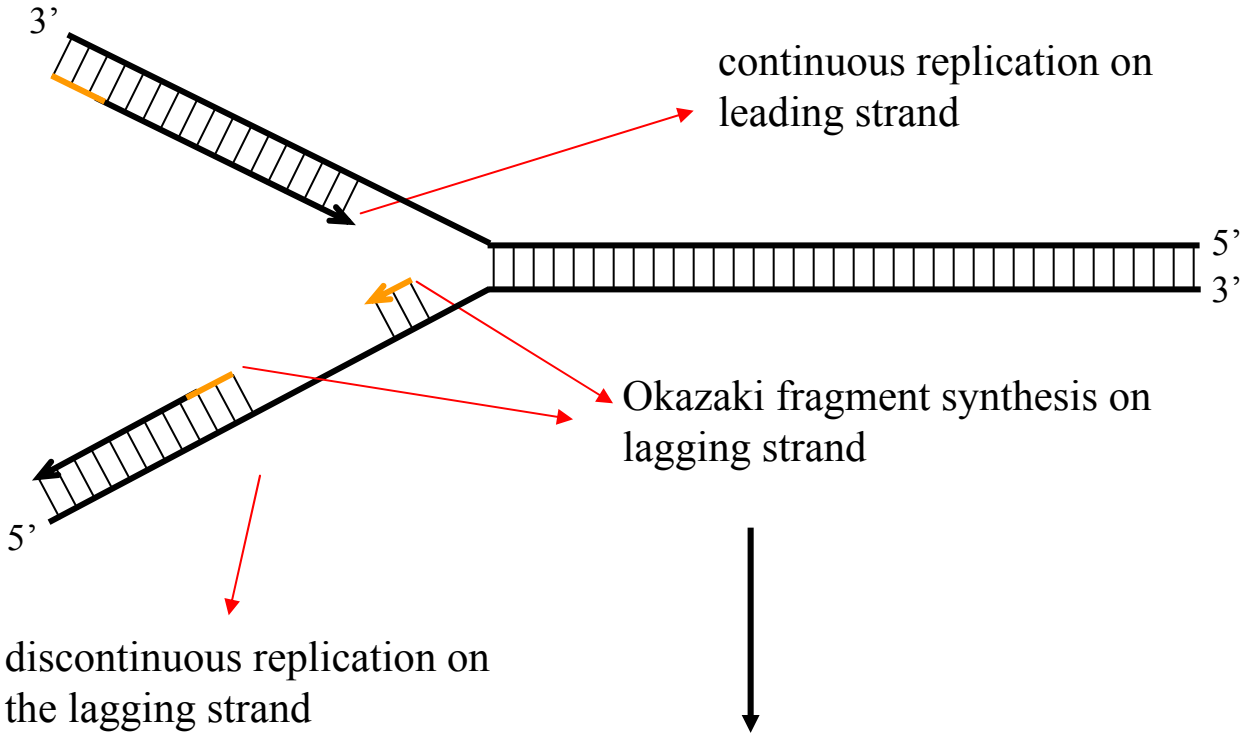
Why

- ✓ Complete DNA replication.
- ✓ Broken chromosomes are unstable. Telomeres protect chromosomes from end to end fusion.

Features

- ✓ The DNA strand running from 5' to 3' from the center toward the end of the chromosomes has more G residues than its complement. The G's are clustered.
- ✓ Telomeres are tandem arrays of $(T/A)_{1-4}\ (G)_{1-8}$. For example (TTAGGG)n in human.
- ✓ The amount of telomeric DNA varies within and between species.

DNA synthesis is semi-discontinuous

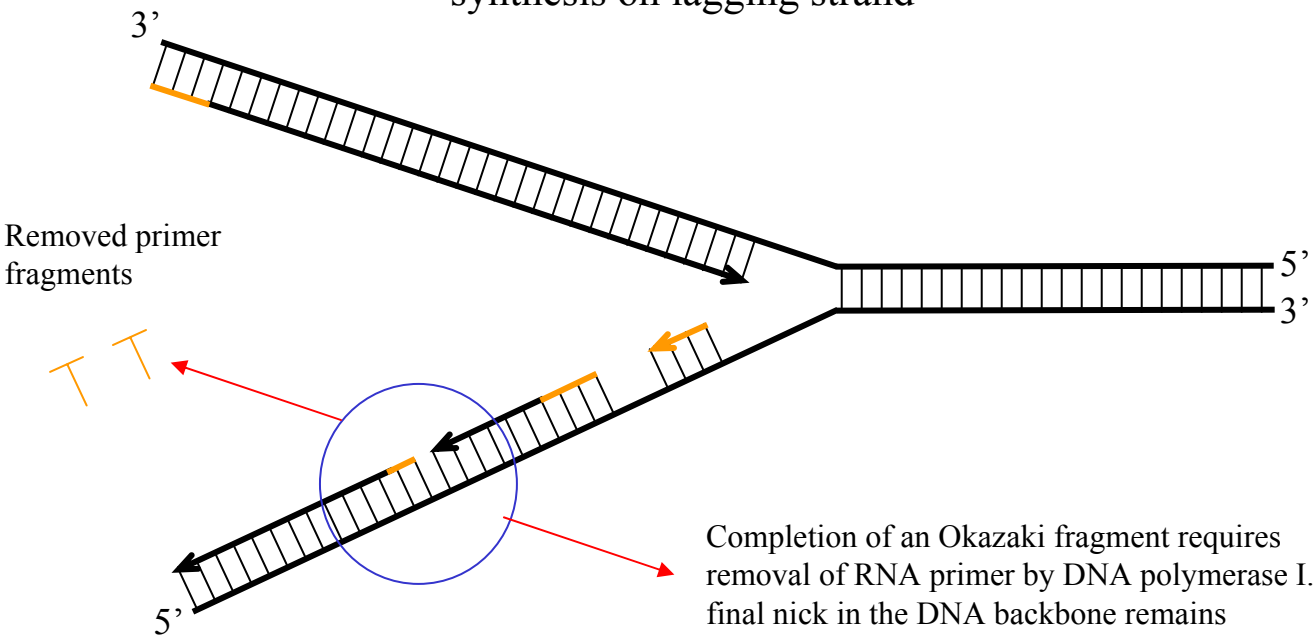


Continuous replication on leading strand

+

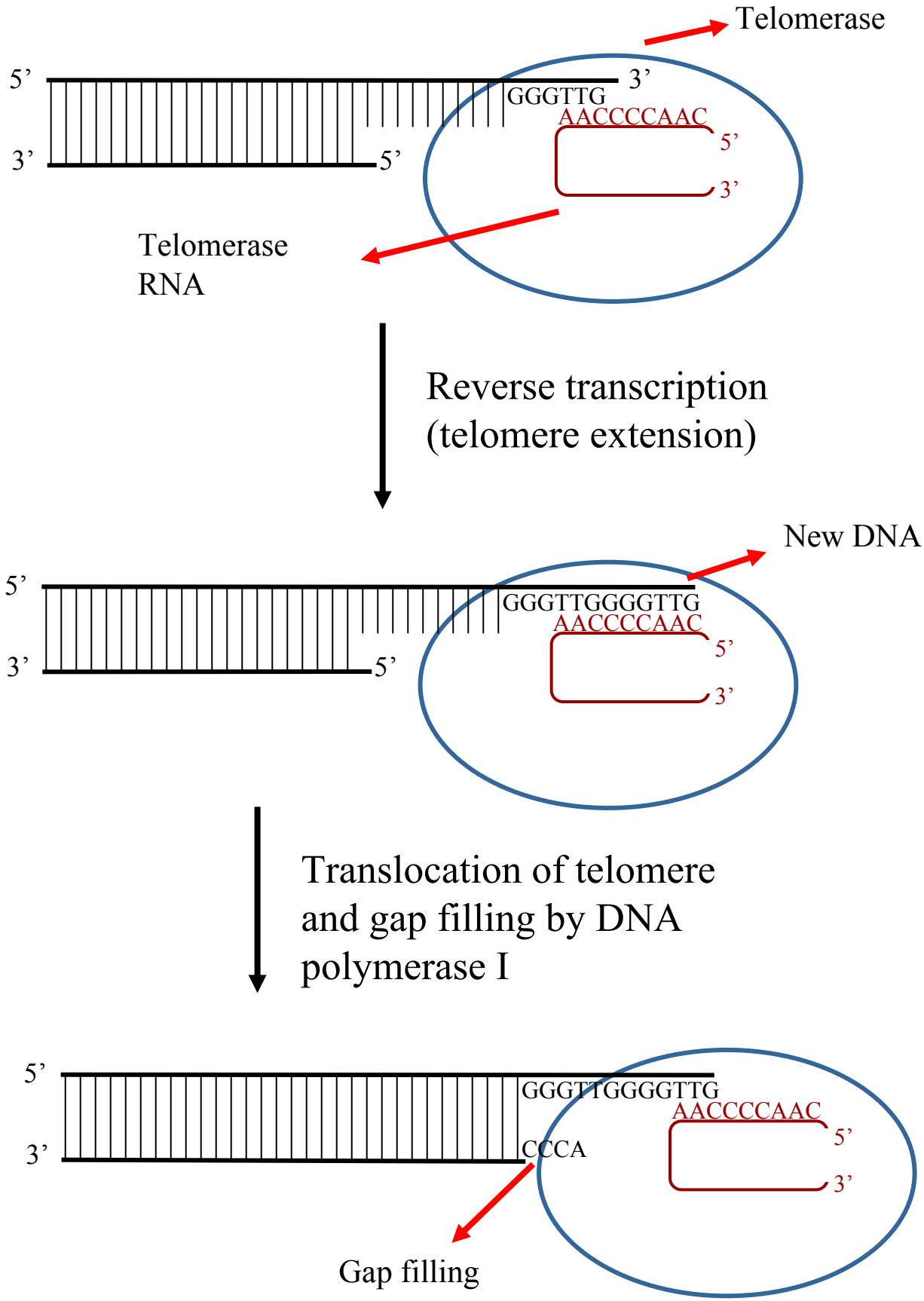
Discontinuous replication on lagging strand

RNA primer formation and elongation by DNA polymerase III result in Okazaki fragment synthesis on lagging strand



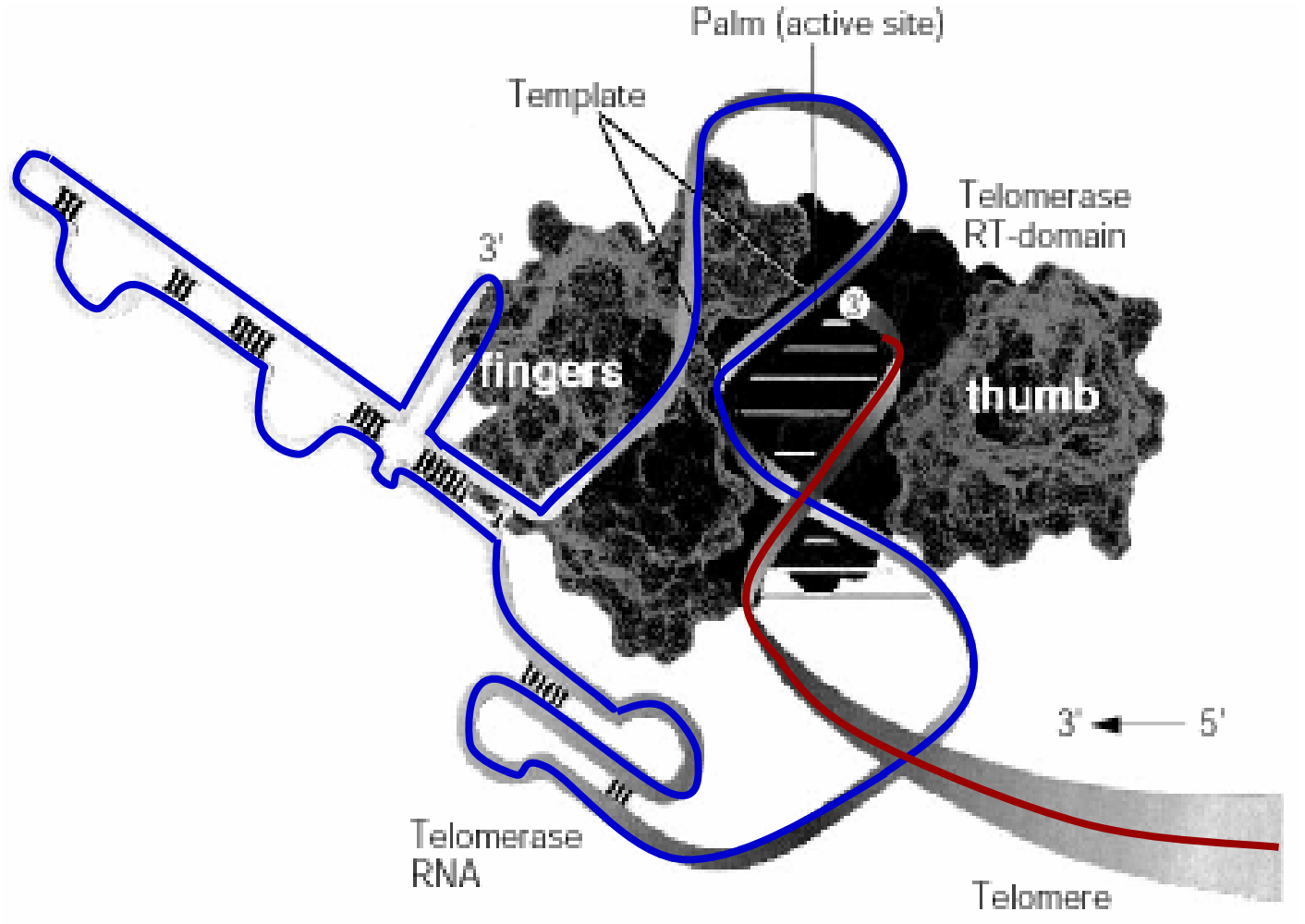
DNA synthesis is semi-discontinuous

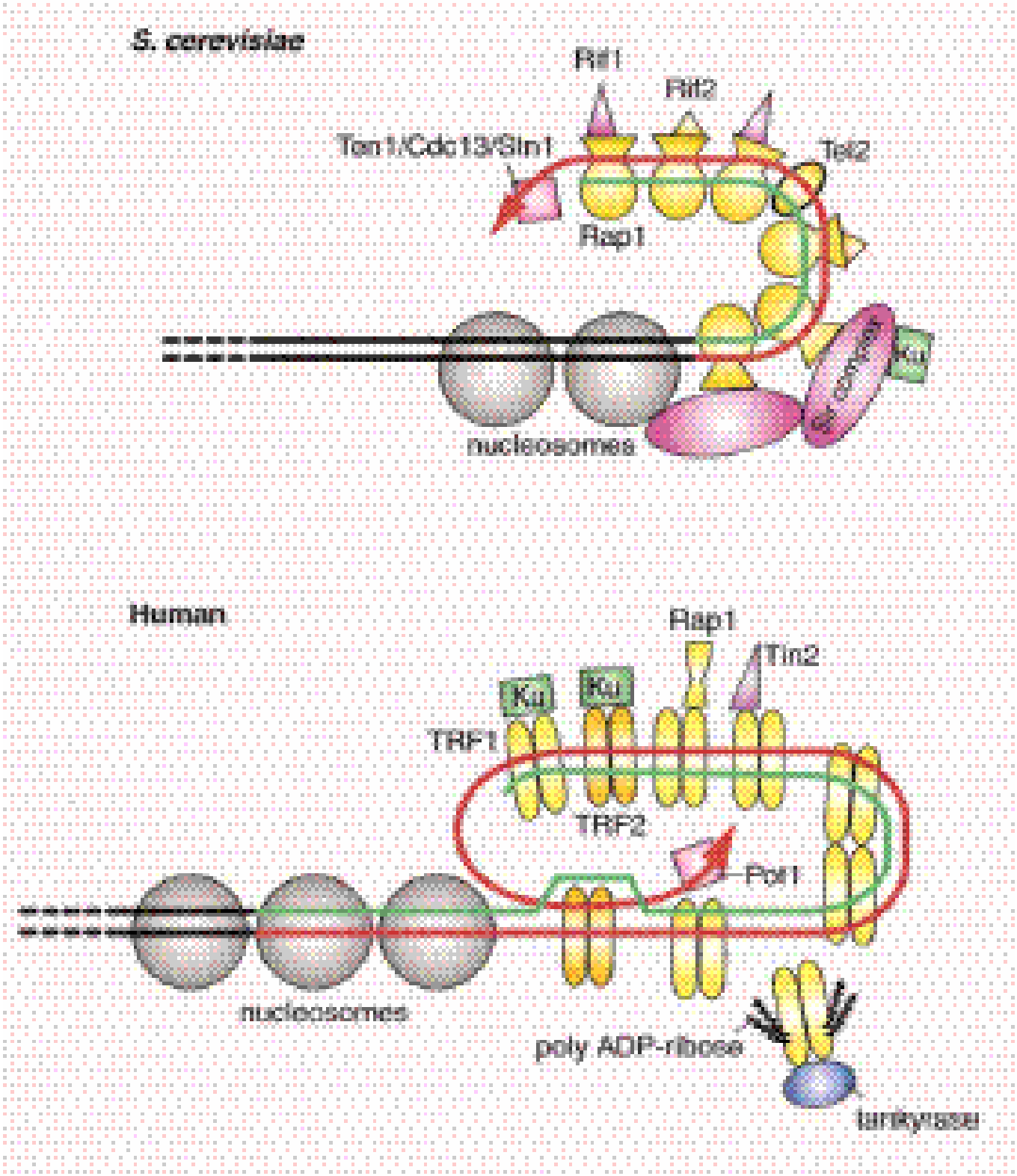
- ✓ DNA polymerase replicates DNA in 5'-3' direction using 8-12 base stretch of RNA to prime DNA synthesis
- ✓ One strand of a linear chromosome will be replicated to the very end, the other will have a 8-12 base gap generated by the removal of the primer.
- ✓ Telomeres act as substrate for replication of the ends and prevent exonucleolytic degradation of chromosomes.

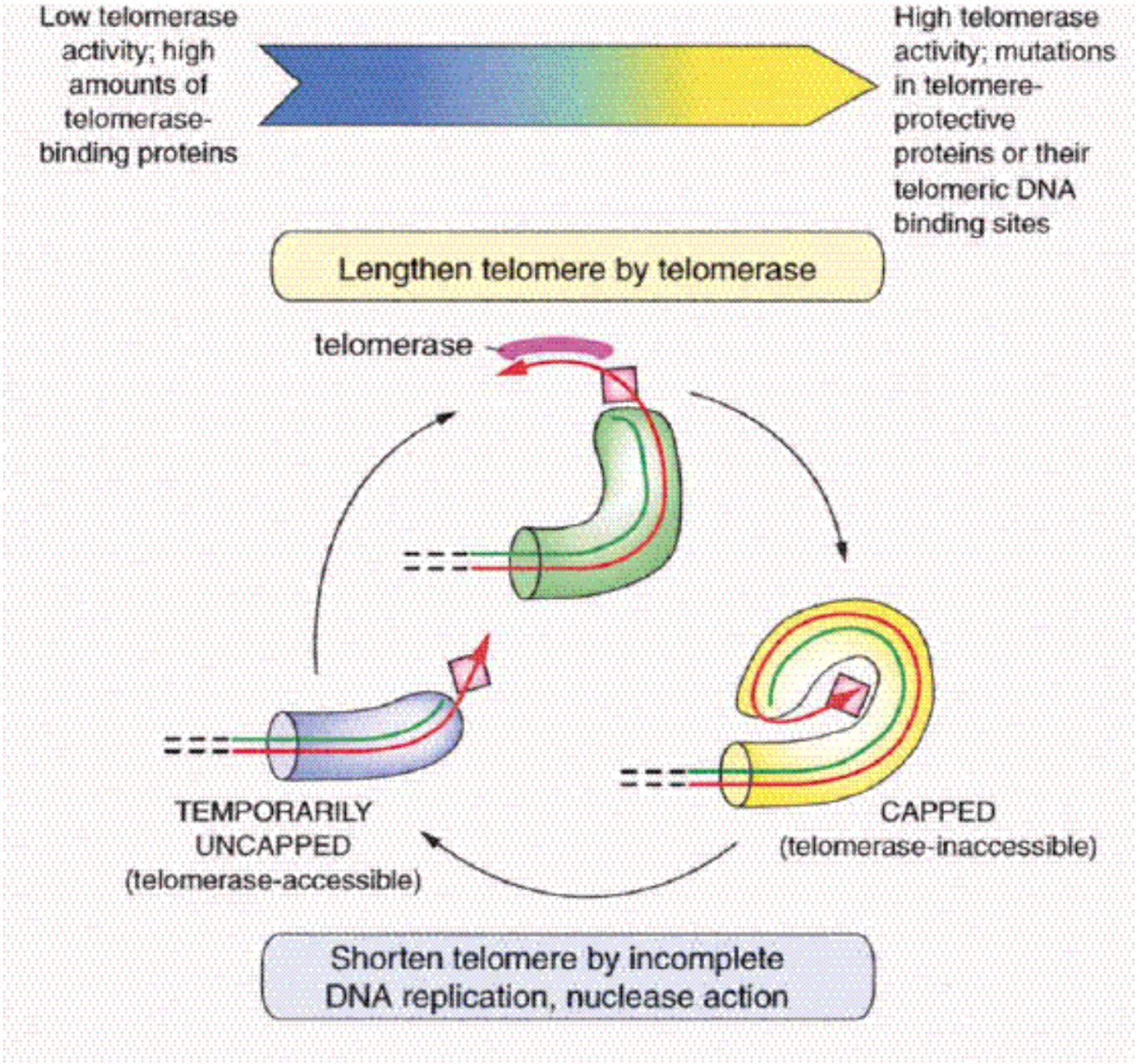

Telomerase
5’
3’
GGGTTG
AACCCCAAC
5’
3’
3’
5’
Telomerase
RNA
Reverse transcription
(telomere extension)
New DNA
5’
3’
GGGTTGGGGTTG
AACCCCAAC
5’
3’
5’
3’
Translocation of telomere
and gap filling by DNA
polymerase I
5’
3’
GGGTTGGGGTTG
AACCCCAAC
CCCA
5’
3’
Gap filling

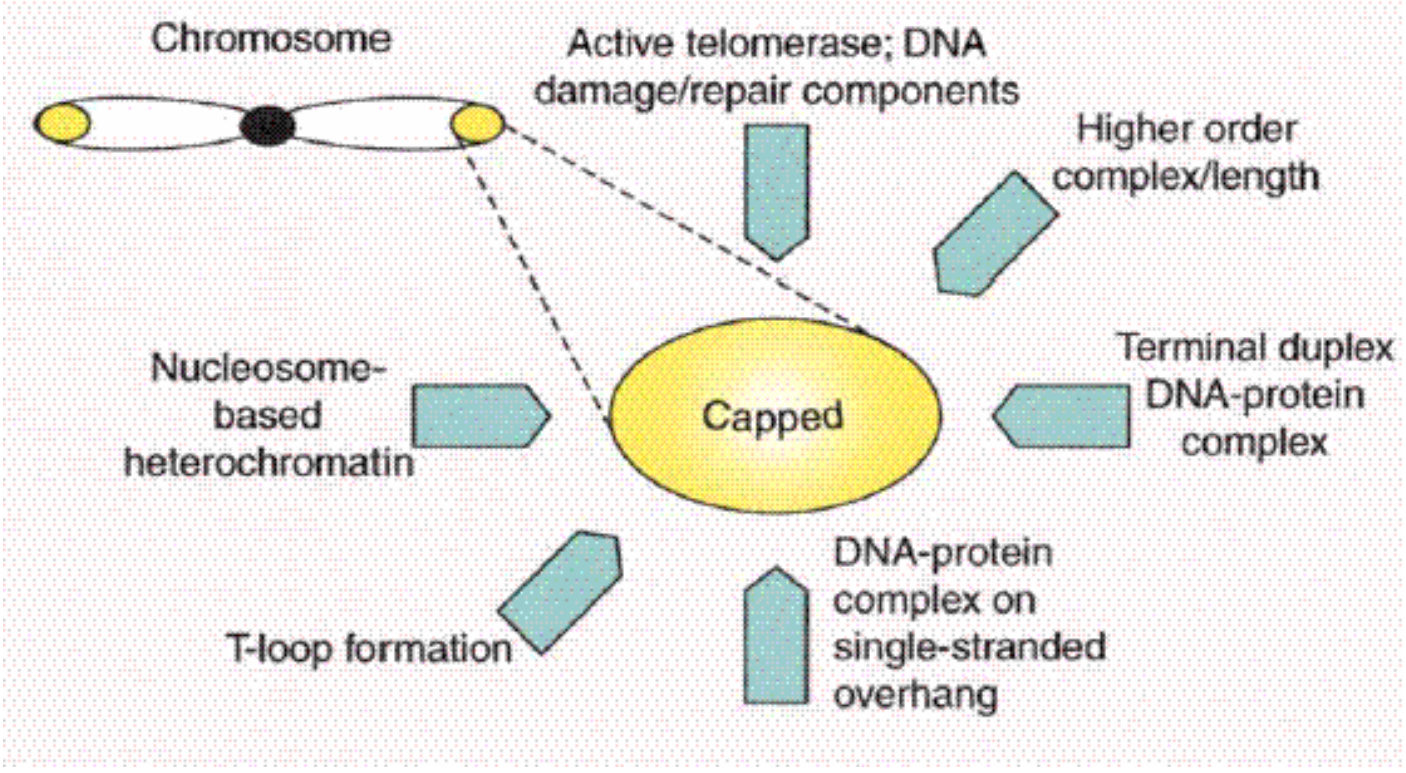
Telomerase

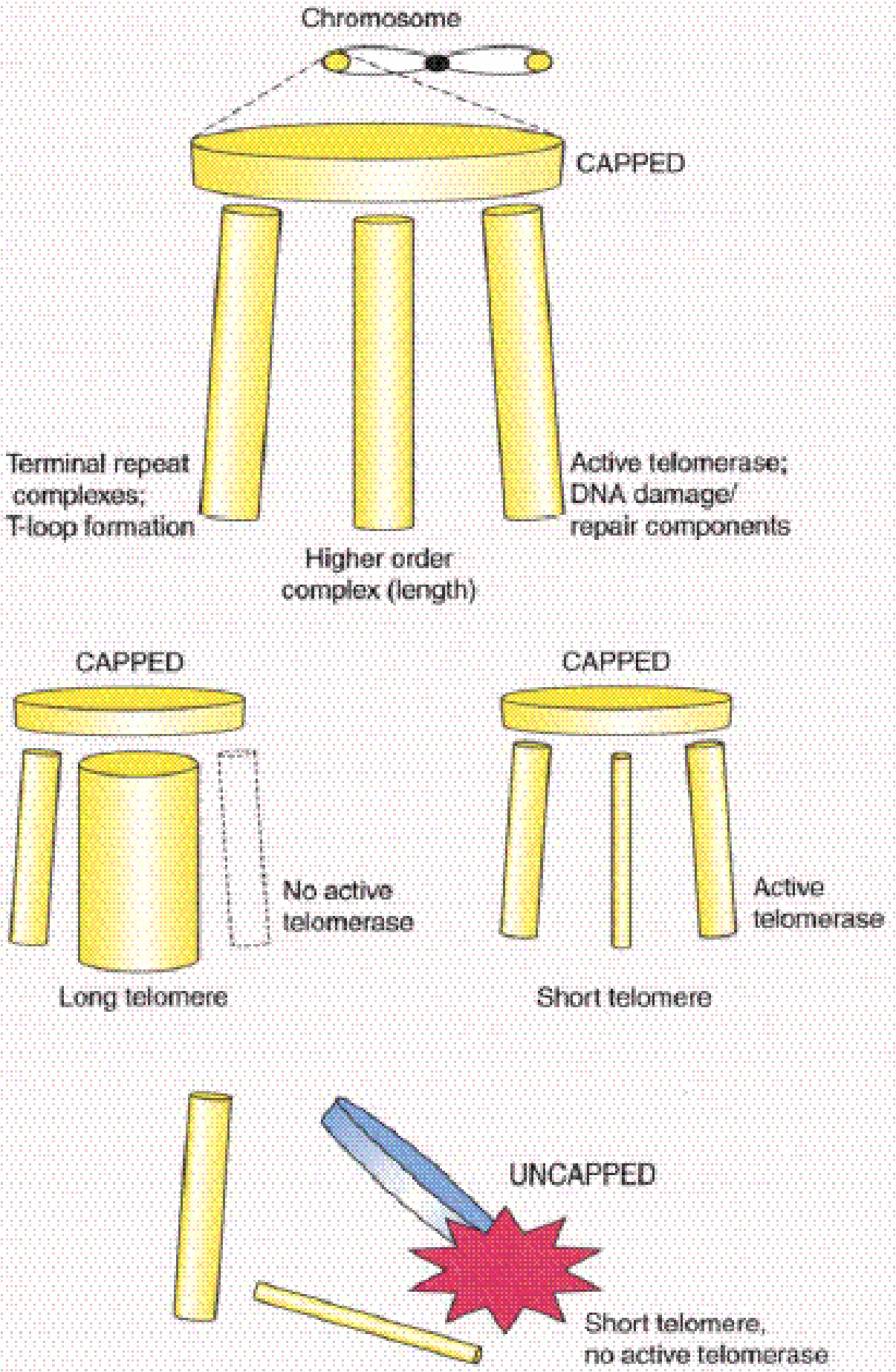
- ✓ Specialized reverse transcriptase
- ✓ Ribonucleoprotein with activity depending on both RNA and protein components.
- ✓ Uses RNA, not DNA to template synthesis of the telomeric DNA
- ✓ The expected product of telomerase mediated replication is a duplex molecule with a single strand G tail
- ✓ Telomerase RNA determines the sequence of telomeric DNA
- ✓ Telomerase independent mechanisms exist for telomere maintenance in humans (no telomerase activity found in somatic cells).


Palm (active site)
Template
Telomerase
RT-domain
3'
fingers
thumb
3'
5'
Telomerase
RNA
Telomere


S. cerevisiae
Rif1
Rif2
Tel2
Tan1/Cdc13/Stn1
Rap1
Ku
nucleosomes
Human
Rap1
Tin2
Ku
Ku
TRF1
TRF2
Pot1
nucleosomes
poly ADP-ribose
tankyrase

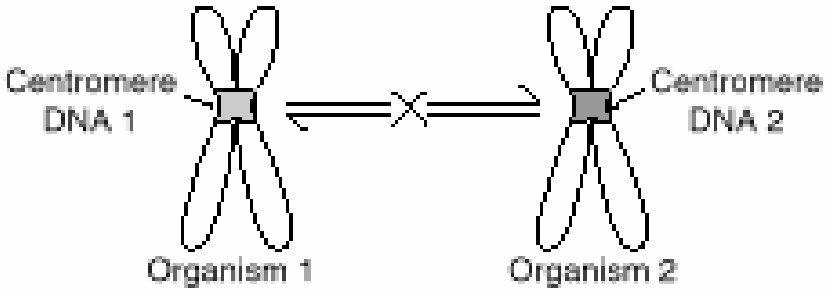
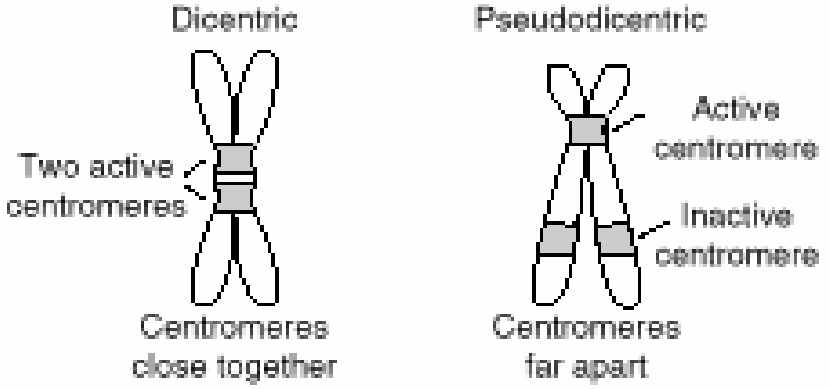
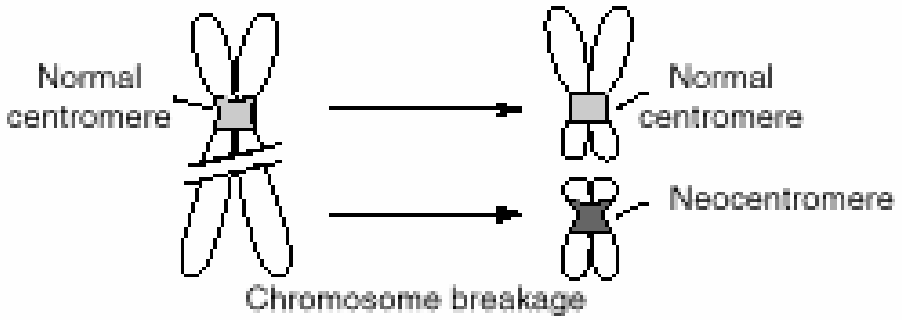
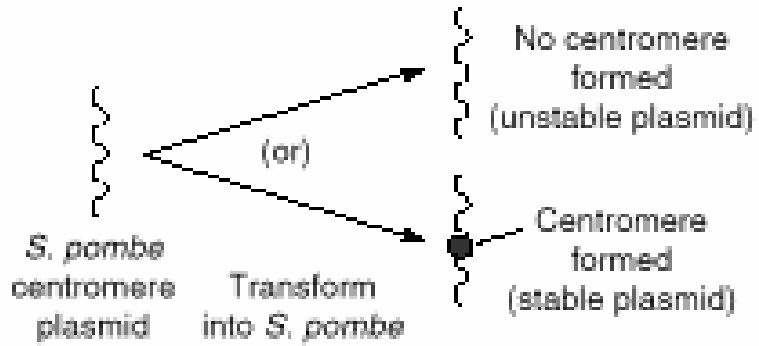

Low telomerase activity; high amounts of telomerase-binding proteins
High telomerase activity; mutations in telomere-protective proteins or their telomeric DNA binding sites
Lengthen telomere by telomerase
telomerase
TEMPORARILY UNCAPPED (telomerase-accessible)
CAPPED (telomerase-inaccessible)
Shorten telomere by incomplete DNA replication, nuclease action

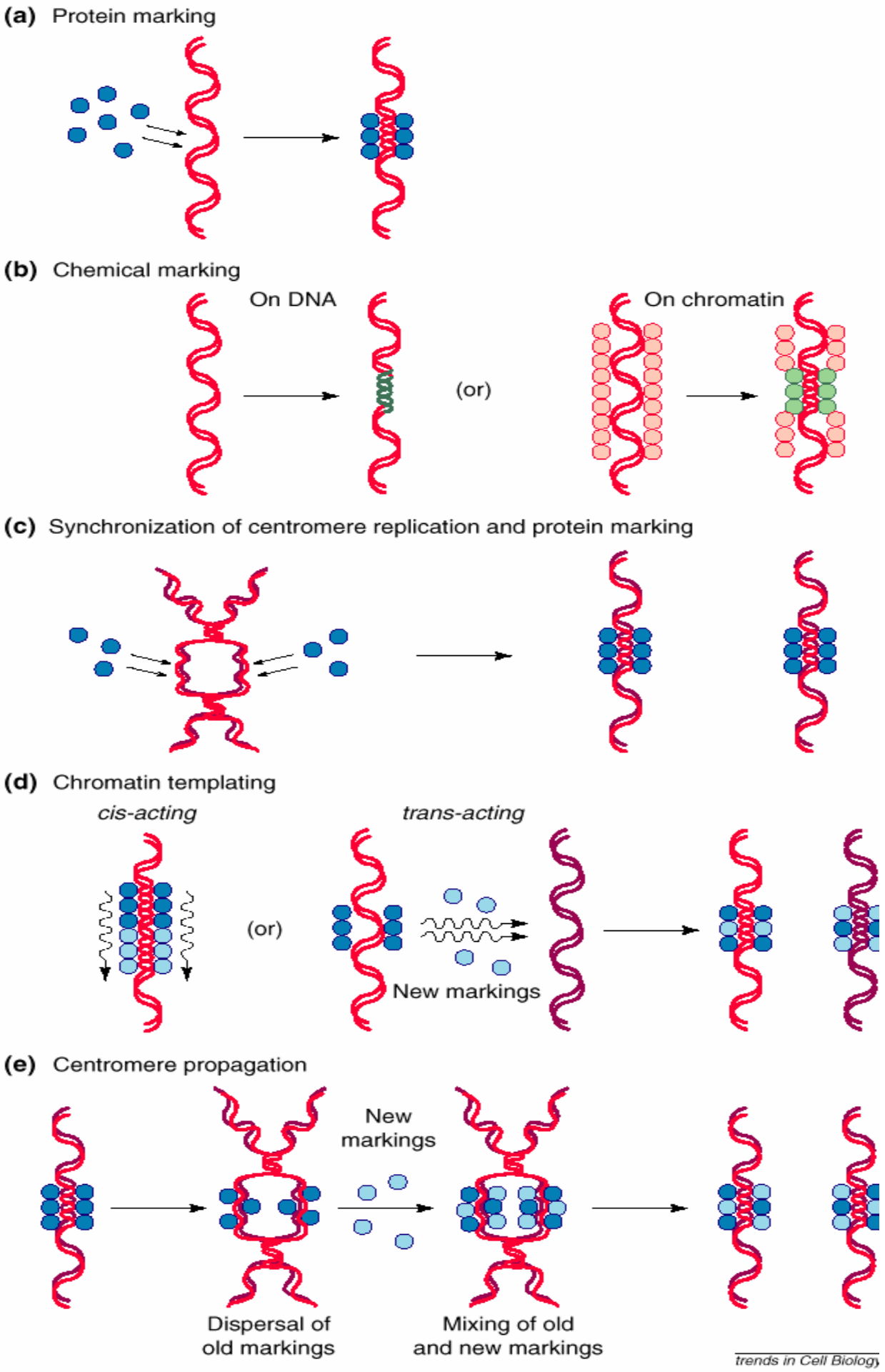

Chromosome
Active telomerase; DNA damage/repair components
Higher order complex/length
Nucleosome-based heterochromatin
Capped
Terminal duplex DNA-protein complex
T-loop formation
DNA-protein complex on single-stranded overhang

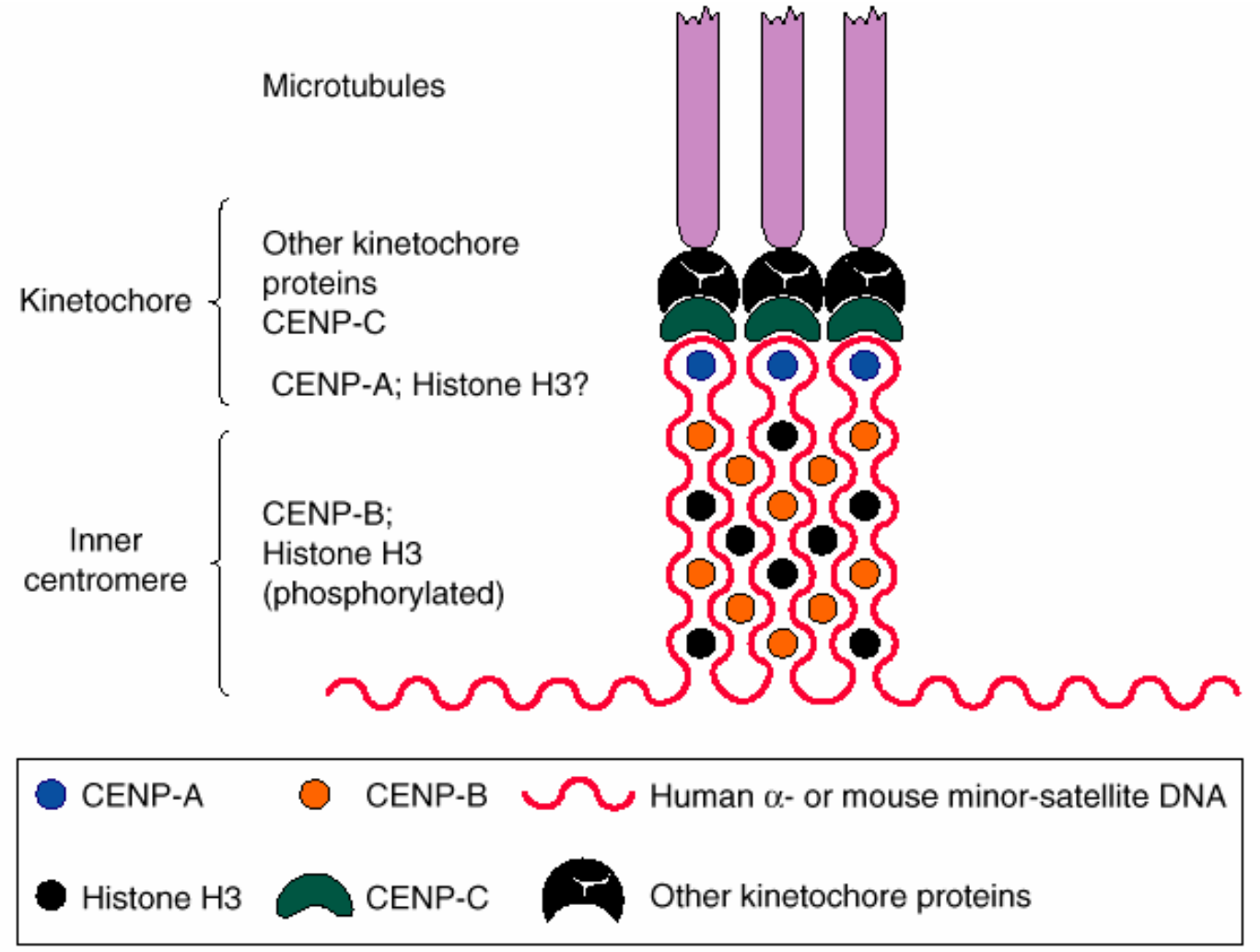

Chromosome
CAPPED
Terminal repeat complexes; T-loop formation
Active telomerase; DNA damage/ repair components
Higher order complex (length)
CAPPED
No active telomerase
Long telomere
CAPPED
Active telomerase
Short telomere
UNCAPPED
Short telomere, no active telomerase

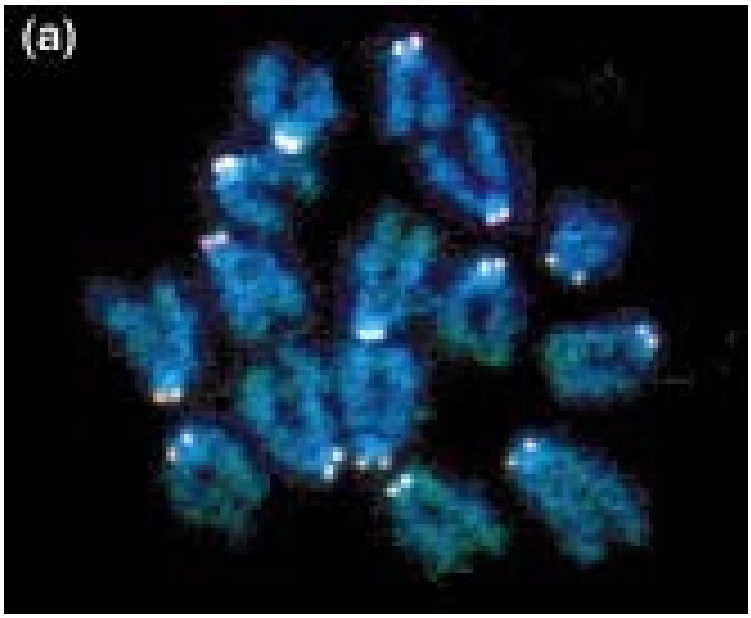
Centromere

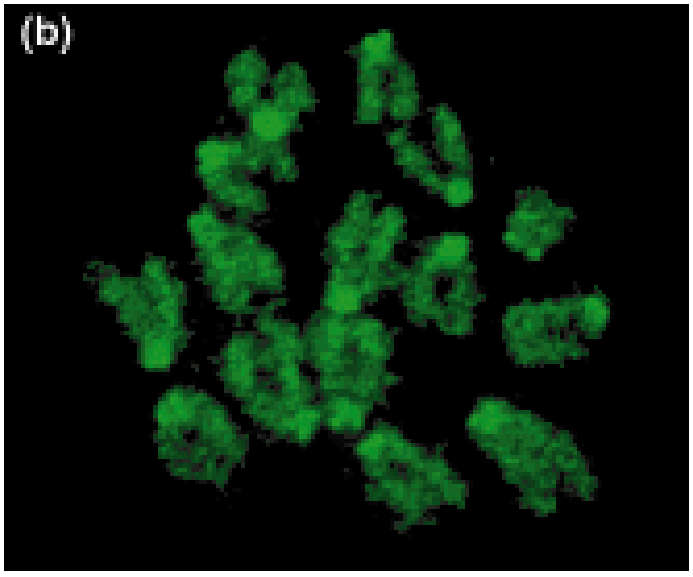
- ✓ CEN locus- DNA sequence that specifies the centromere location
- ✓ Site of formation of the kinetochore
- ✓ Final locus of sister chromatid pairing in mitosis and meiosis
- ✓ Cell cycle checkpoint control
- ✓ DNA contains repetitive sequences
- ✓ Centromeric proteins have been identified which are associated with DNA in the centromere region (CENP-A, B, C and D).

Observation	Mechanistic implication
(a) Centromere sequences are not conserved	
	Recognition of widely different centromeric repeat sequences
(b) Dicentrics/pseudodicentrics with one or two active centromeres	
	Normal centromeric DNA may not form kinetochore Epigenetic influence is distance sensitive
(c) Neocentromeres can form from non-centromeric DNA	
	Recognition of a variety of genomic DNA sequences
(d) Variable centromere competence on the same DNA	
	Sequence-independent centromerization

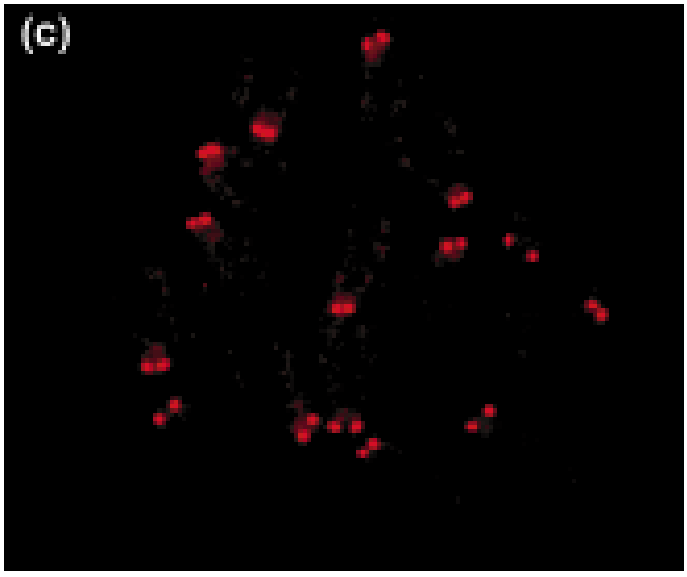

(a) Protein marking
(b) Chemical marking
On DNA
(or)
On chromatin
(c) Synchronization of centromere replication and protein marking
(d) Chromatin templating
cis-acting
(or)
trans-acting
New markings
(e) Centromere propagation
New markings
Dispersal of old markings
Mixing of old and new markings
trends in Cell Biology


Microtubules
Kinetochore
Other kinetochore proteins
CENP-C
CENP-A; Histone H3?
Inner centromere
CENP-B; Histone H3 (phosphorylated)
CENP-A
CENP-B
Human α- or mouse minor-satellite DNA
Histone H3
CENP-C
Other kinetochore proteins





Poly (ADP) polymerase (PARP)



CENP-A / CENP-B