Chapter 11

The Eukaryotic Chromosome

Sections to study

11.1 Chromosomal DNA and proteins

11.2 Chromosome structure and compaction

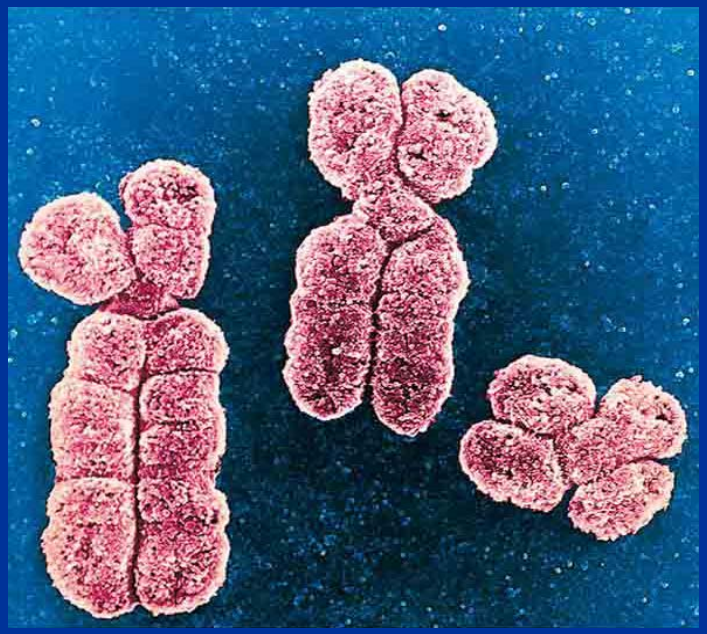
11.3 Chromosomal packaging and gene expression

11.4 Replication of eukaryotic chromosomes

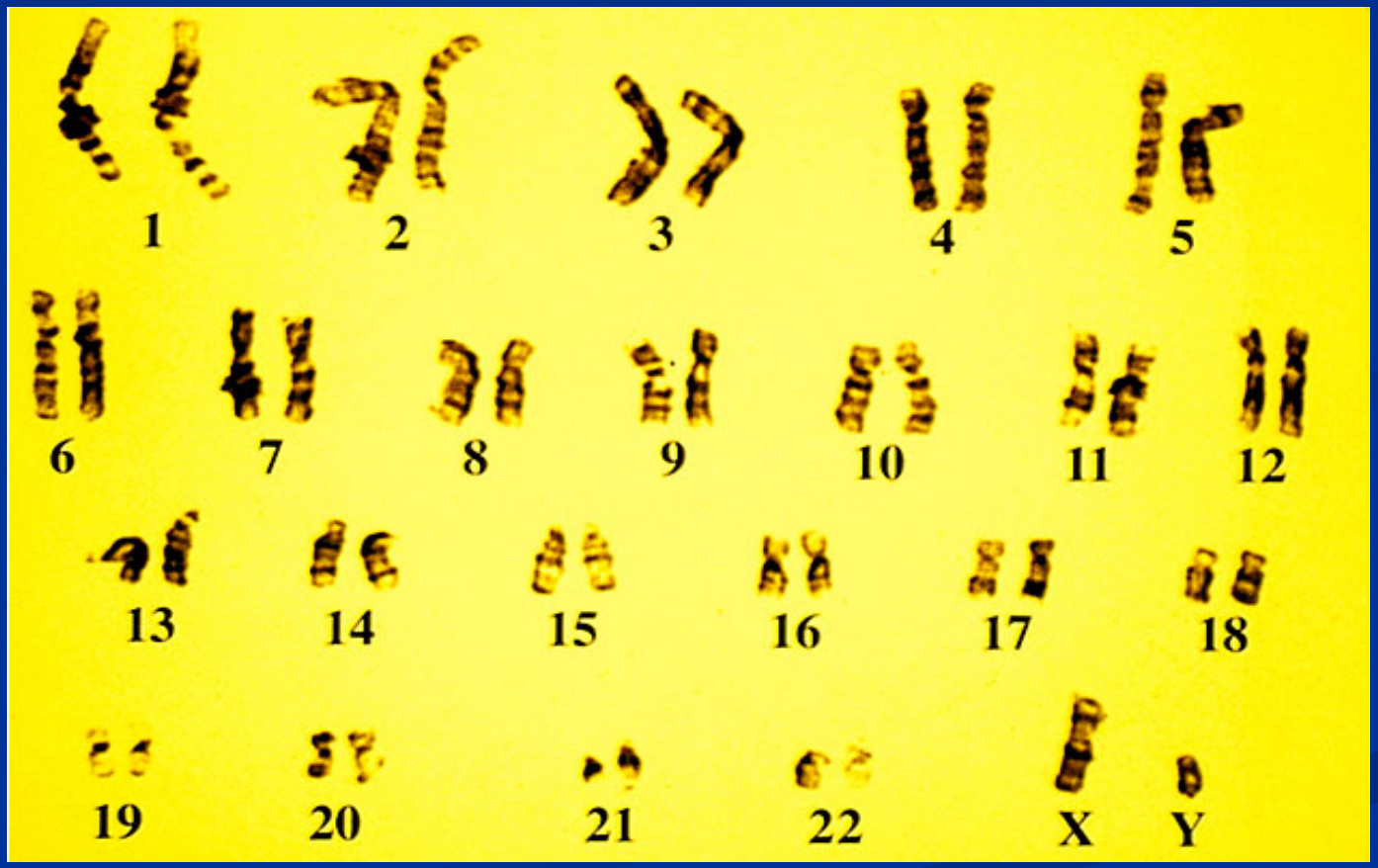
11.5 Chromosome segregation

11.6 Artificial chromosomes

Chromosome − The self-replicating genetic structures of cells containing the DNA that carries in its nucleotide sequence the linear array of genes.

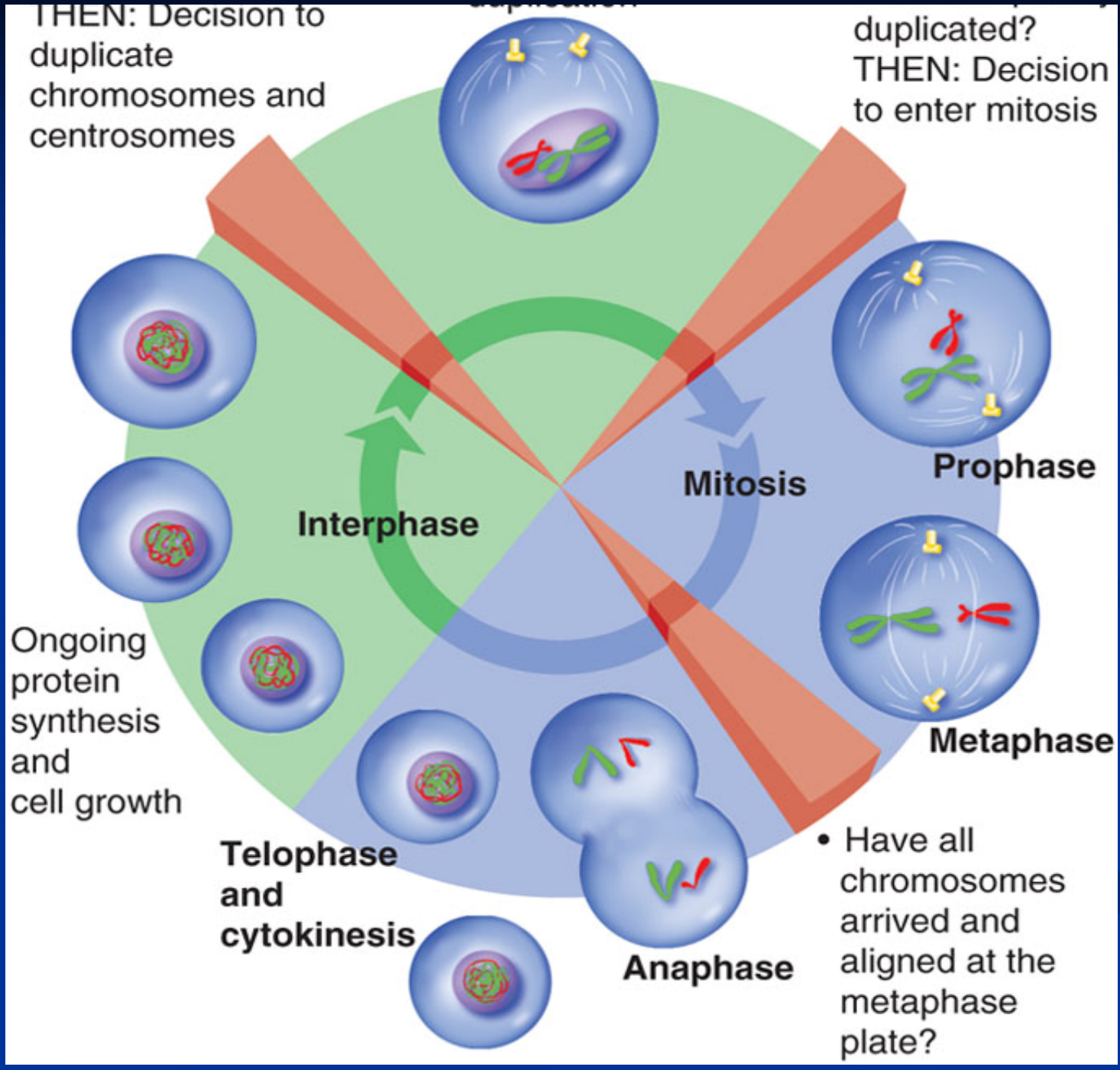


Human chromosomes

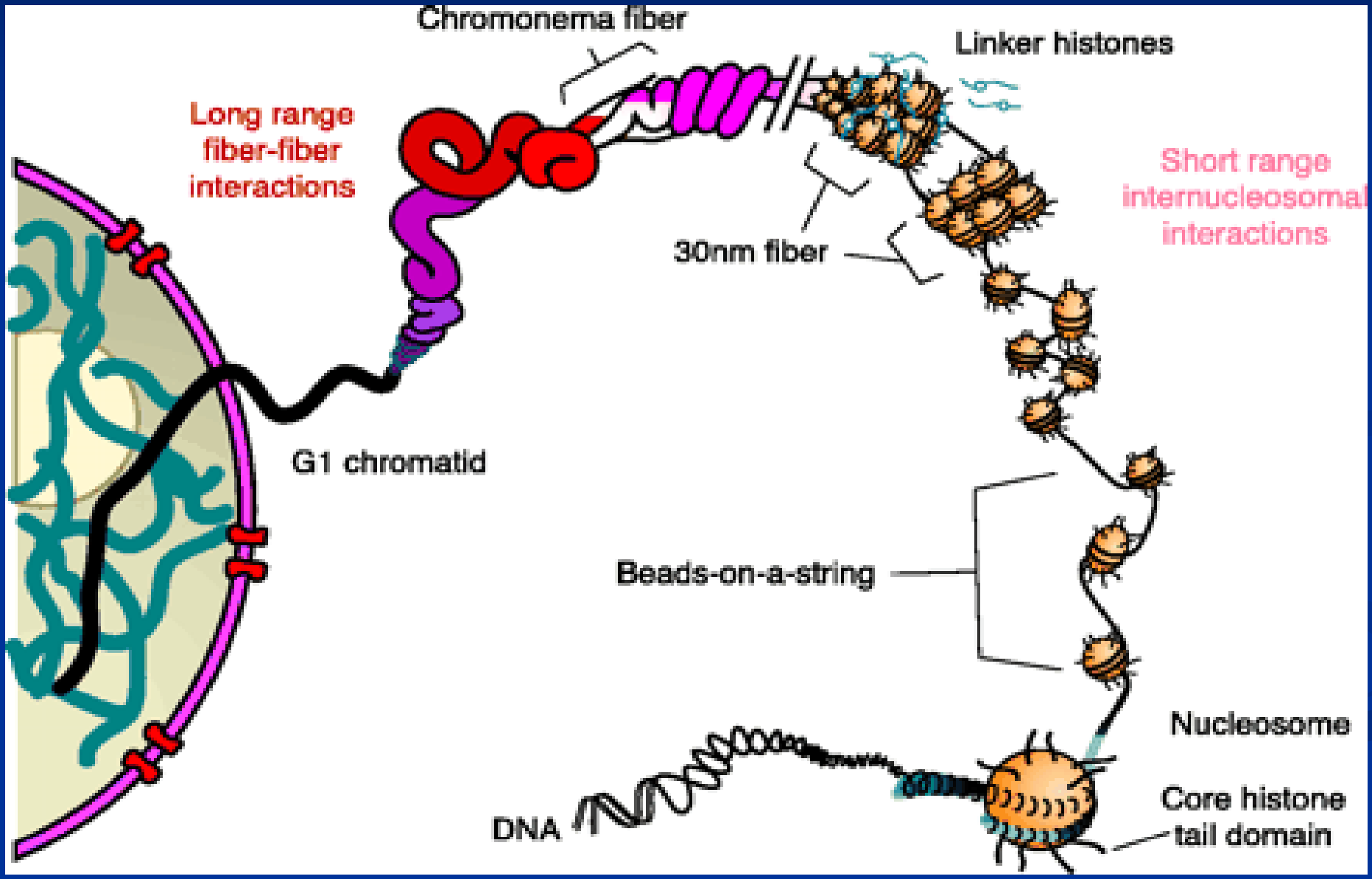


Human male karyotype

Chromosomes change shape and position during the cell cycle.



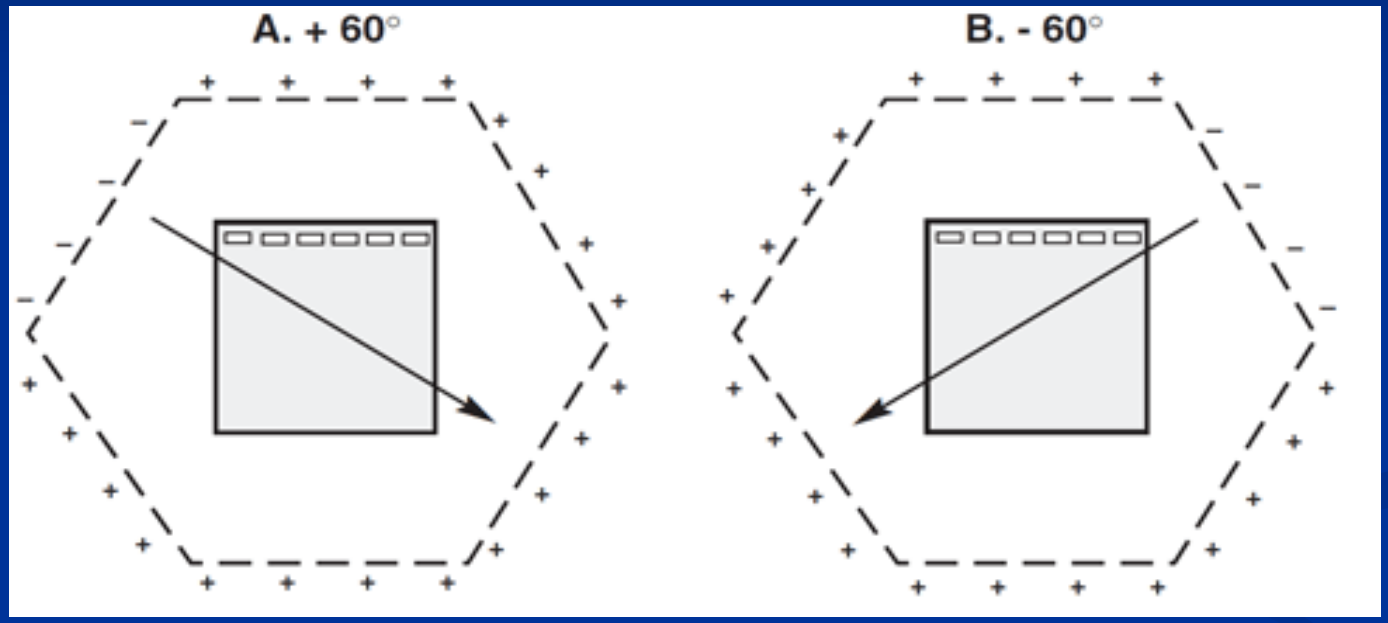
Chromatin（染色质）– The generic term for any complex of DNA and protein found in a cell's nucleus.

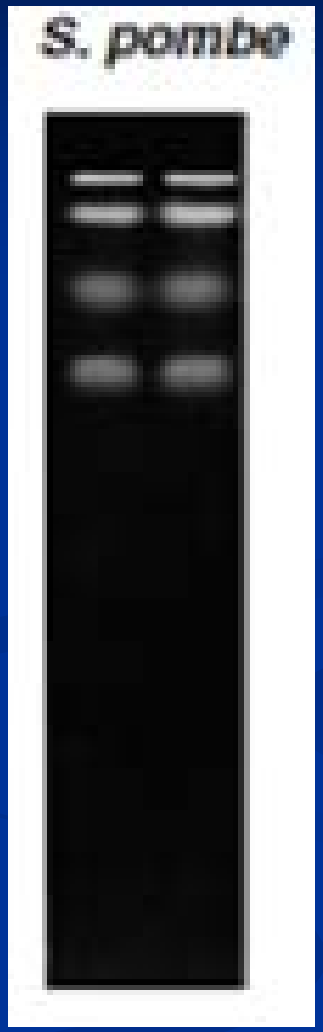


11.1 Chromosomal DNA and proteins

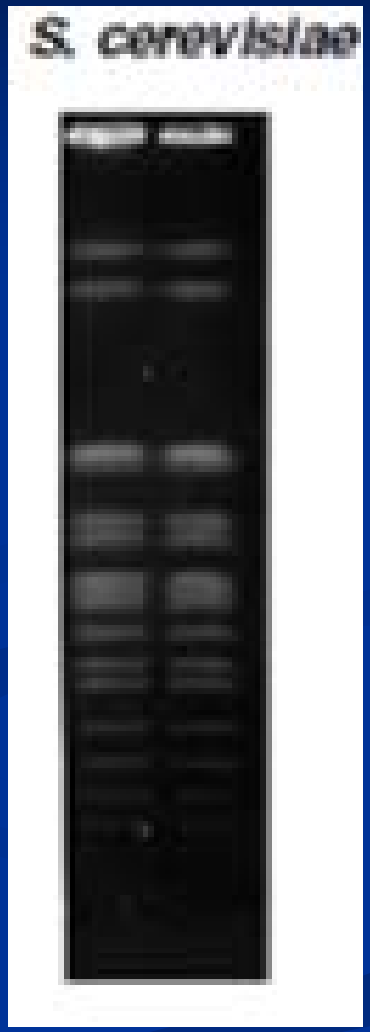
- The chromatin is roughly 1/3 DNA, 1/3 histones, and 1/3 nonhistone proteins by weight.
- Each chromosome packages a single long molecule of DNA.
  - Studies examine stretching and recoiling of chromosomes. Longer pieces recoil more slowly than shorter pieces.
  - Pulse field gel electrophoresis (PFGE)
    - Separates large pieces of DNA – number and sizes correspond to number and sizes expected if each chromosome contains a single piece of DNA

Pulse field gel electrophoresis (PFGE)





3 chr
2,450-5,580 kb



16 chr
300-2,000 kb each

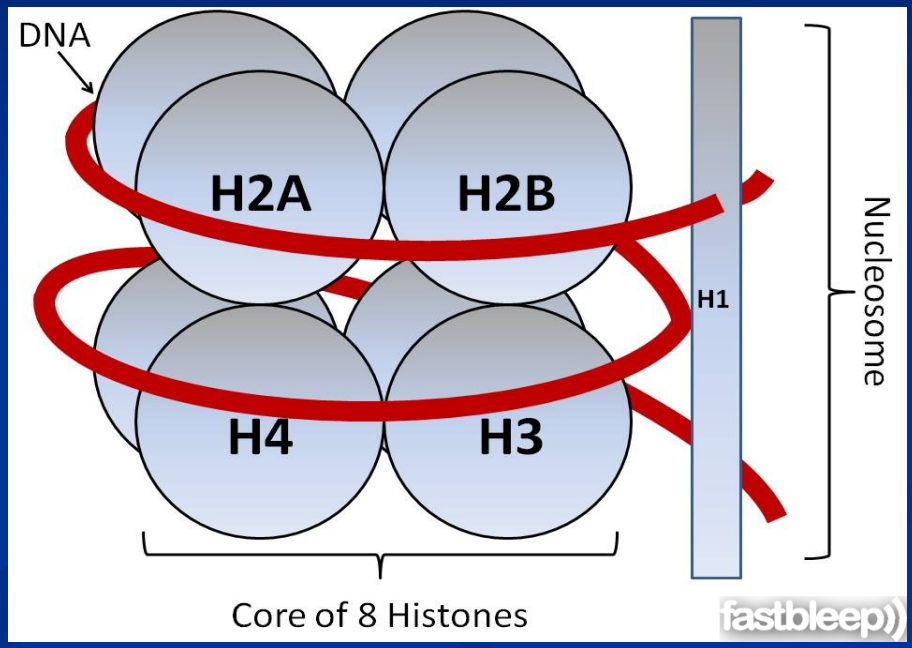
Protein components of chromosomes

- Histone proteins abound the chromatin of all eukaryotic cells.
  - Histones – small proteins with basic, positively charged amino acids lysine and arginine.
  - Bind to and neutralize negatively charged DNA.
  - Make up half of all chromatin protein by weight.

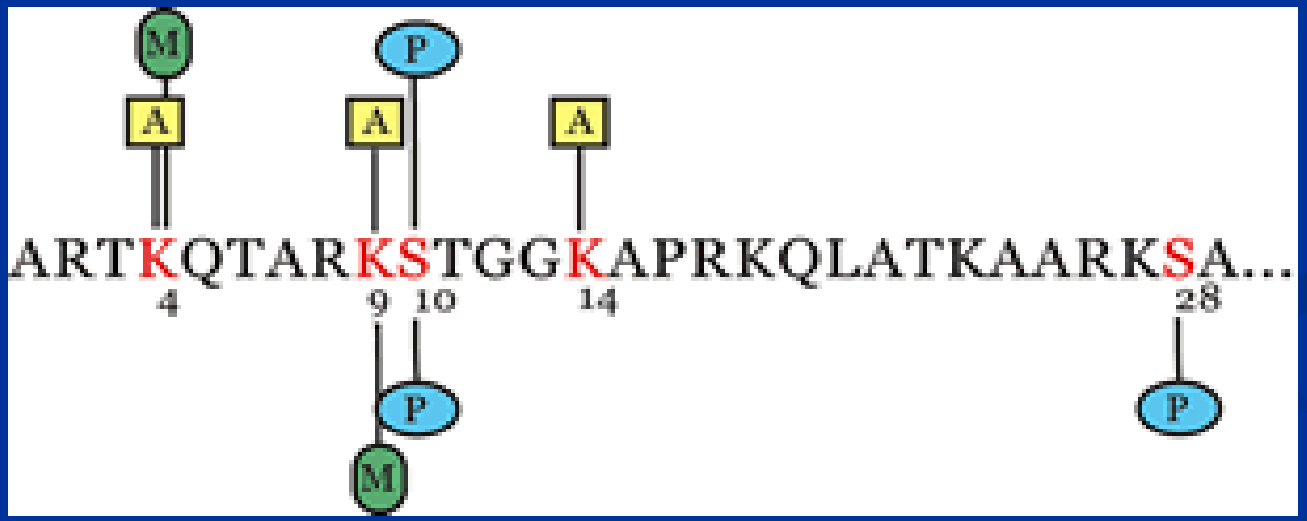
```
  1  MARTKQTARK STGGKAPRKQ LASKAARKSA PSTGGVKKPH RYKPGTVALR
 51  EIRRFQKSTE LLIRKLPFQR LVREIAQDFK TDLRFQSSAI GALQESVEAY
101  LVSLFEDTNL AAIHAKRVTI QKKDIKLARR LRGERS*
```

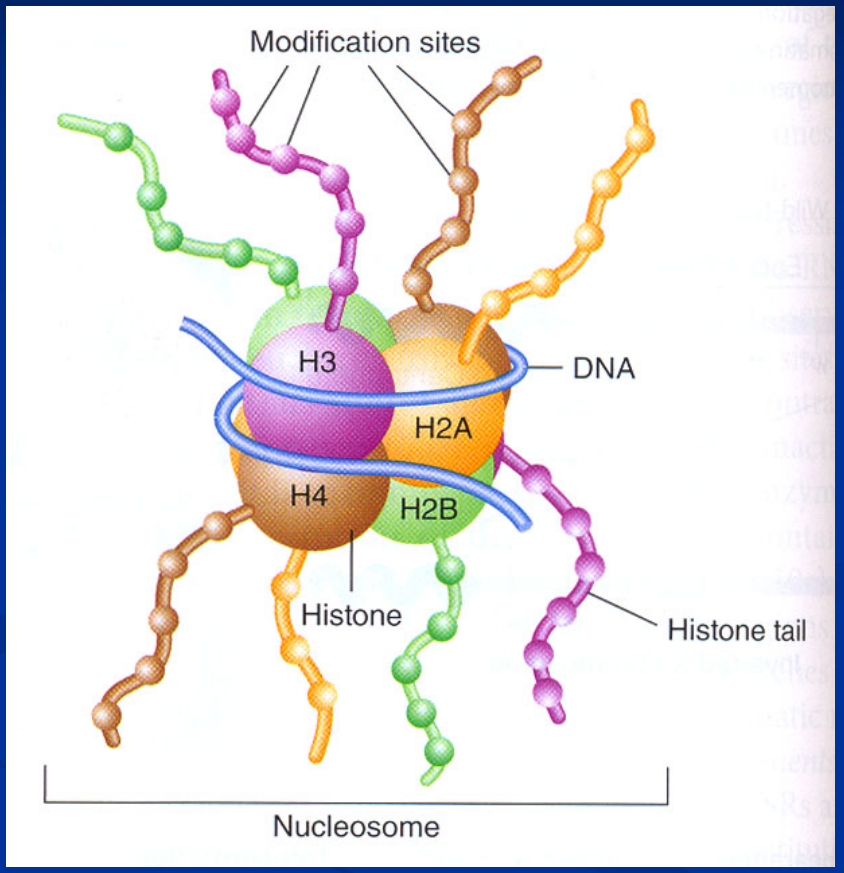
Yeast histone H3 (Hht1)

- **Histone proteins**
  - **Five types: H1, H2A, H2B, H3, and H4.**
  - **Core histones make up nucleosome: H2A, H2B, H3, and H4.**
  - **DNA and histone synthesis regulation correlate timing so both are synthesized together.**
  - **Evolutionarily conserved.**



- **Post-translational modification of histones**
  - **Acetylation**
  - **Methylation**
  - **Phosphorylation**



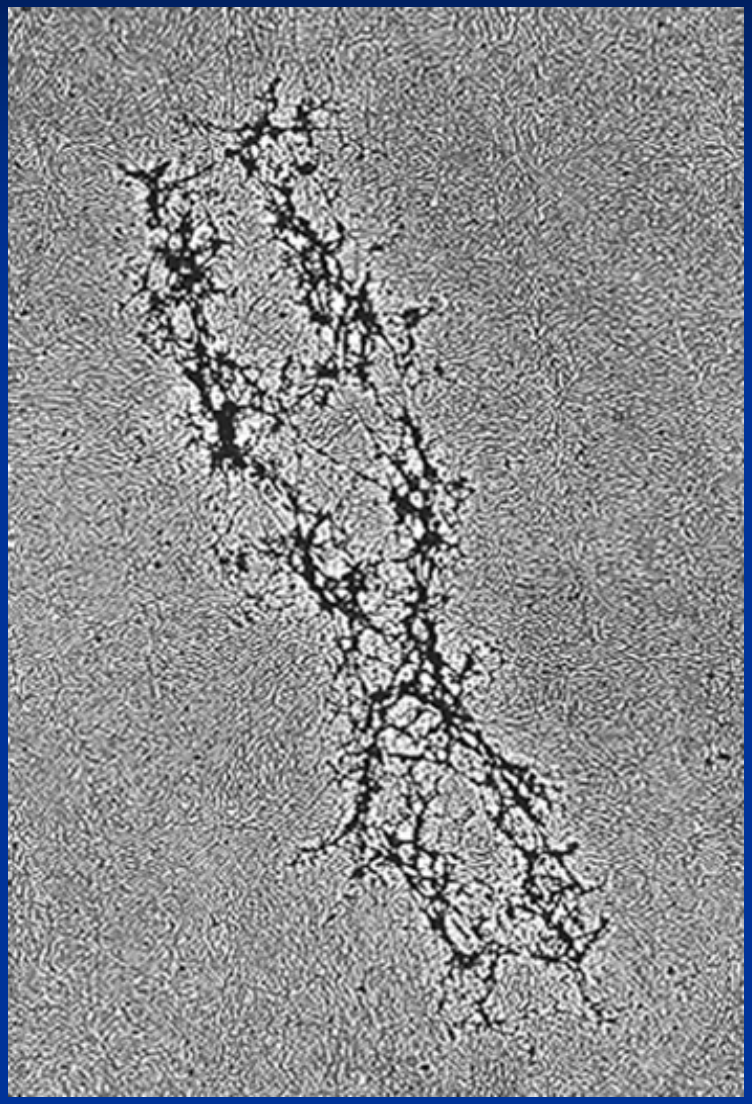


Possible modification of amino acids at the N-terminus of yeast histone H3

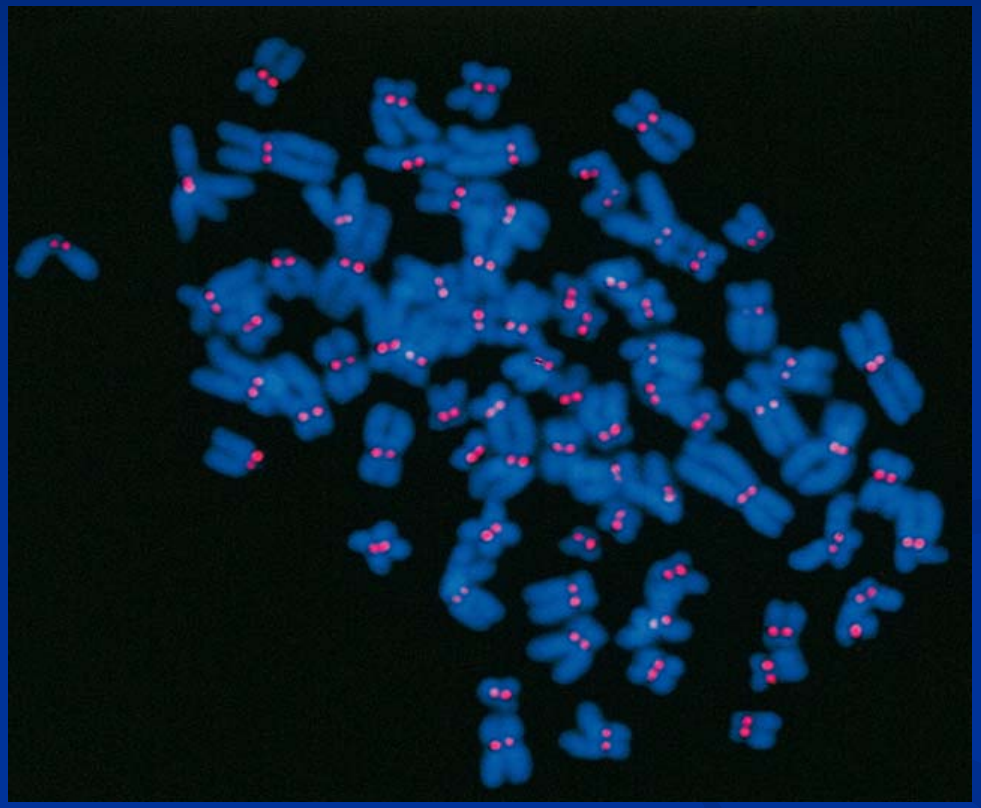
Fig. 11.13

- **Nonhistone proteins are a heterogeneous group.**
  - Half of proteins in chromatin are nonhistones.
  - Large number of nonhistone proteins, 200 – 2,000,000 molecules per diploid genome.
  - Large variety of functions
    - Scaffold – backbone of chromosome
    - DNA replications – e.g., DNA polymerases
    - Chromosome segregation – e.g., motor proteins of kinetochores
    - Transcriptional regulation – largest group regulates transcription during gene expression (5,000 – 10,000 proteins in mammals)
  - Occur in different amounts in different tissues because of variety of function.

Nonhistone proteins have diverse functions



Human chromosome treated with detergent

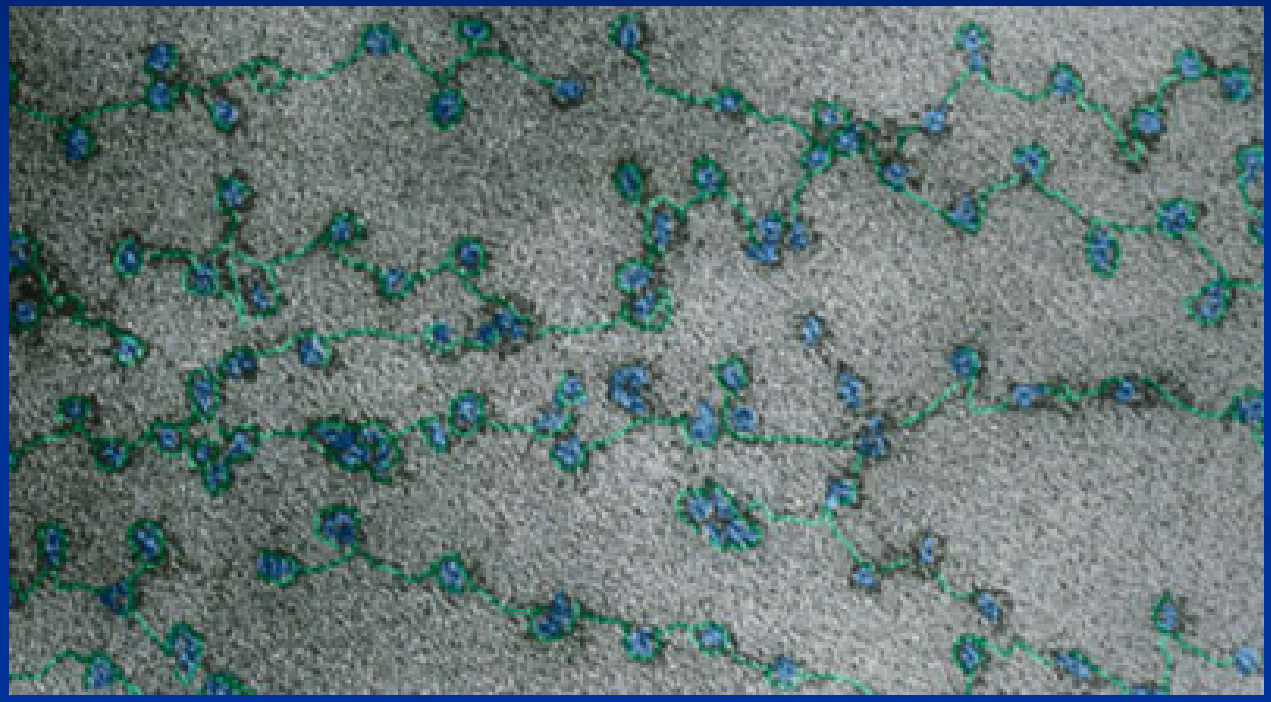


CENP-E staining

Fig. 11.1, 11.2

11.2 Chromosome structure and compaction

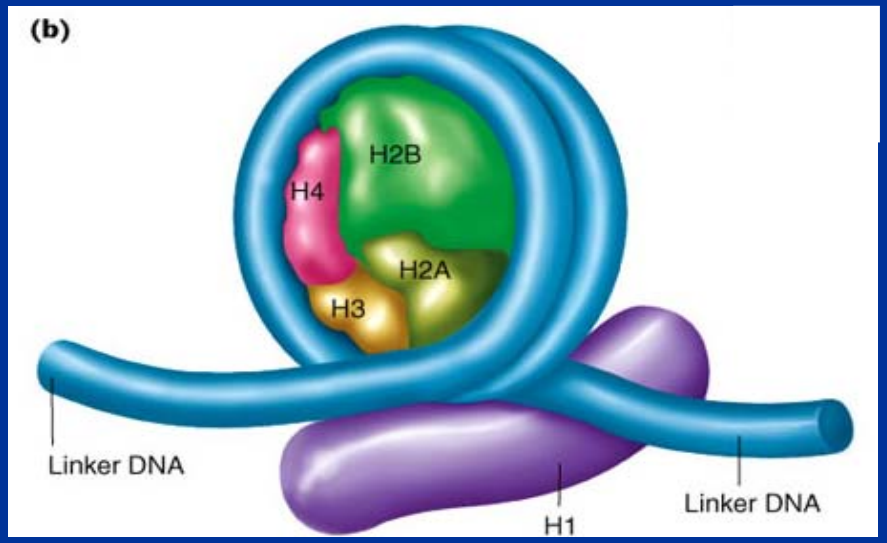
- The nucleosome is the fundamental unit of chromosomal packaging.
- Chromatin fibers with beads having diameter of about 100 Å and strings having diameter of 20 Å.

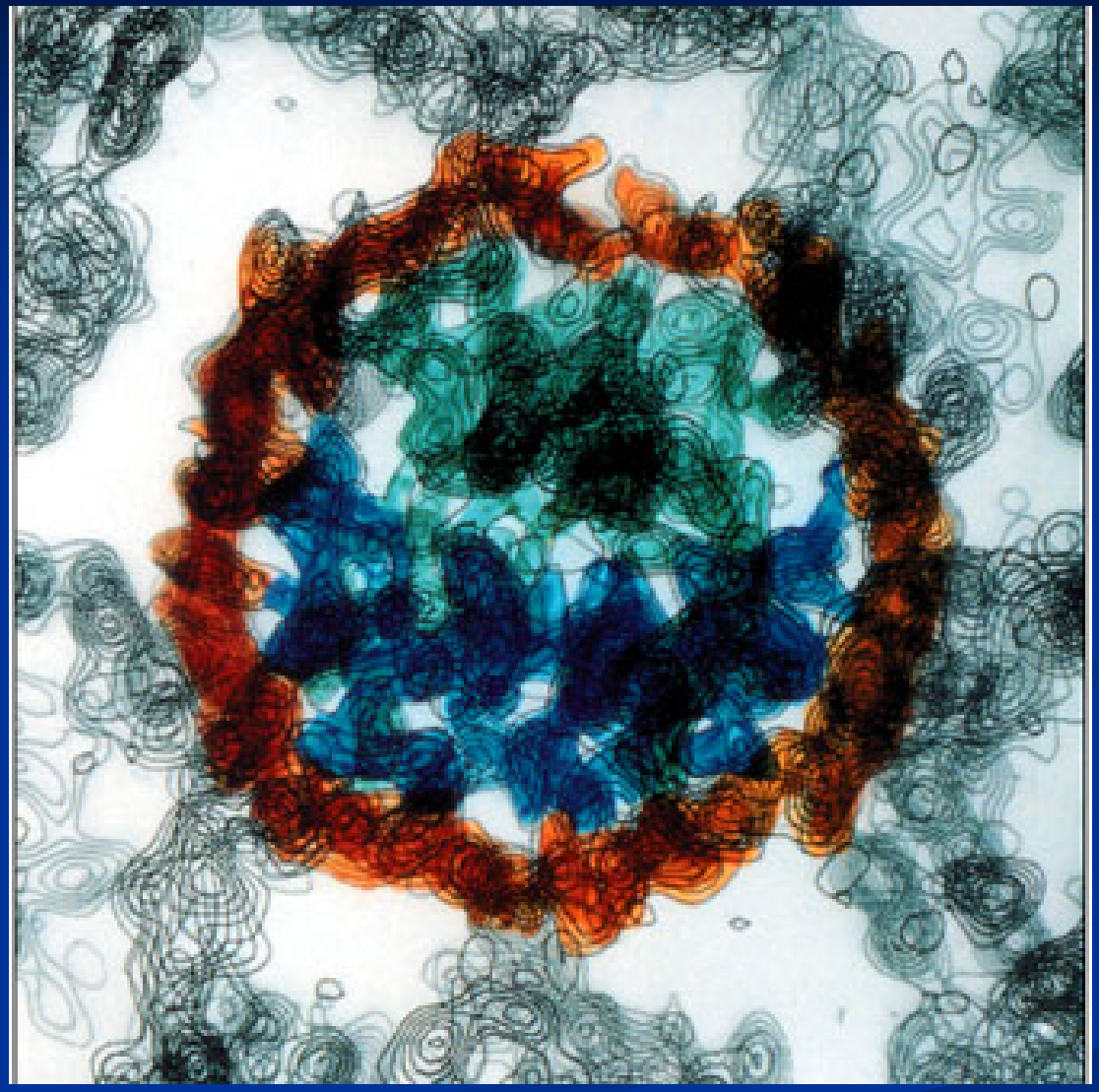


Electron microscopic pictures of nucleosomes (chicken cells)

Fig. 11.3

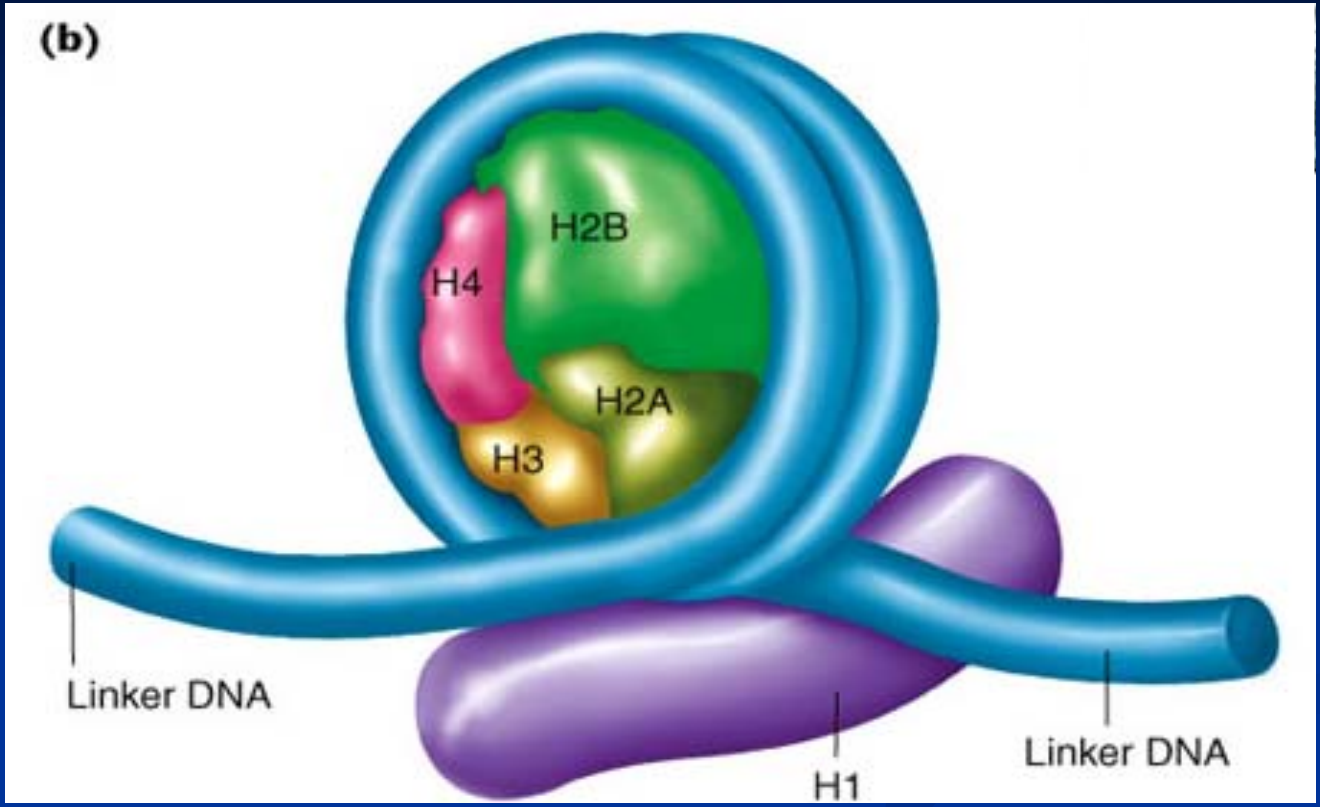
- **X-ray diffraction analysis**
  - DNA does not coil smoothly.
  - Base sequences dictate preferred nucleosome positions along DNA.





X ray crystallography of nucleosome structure

Fig. 11.4

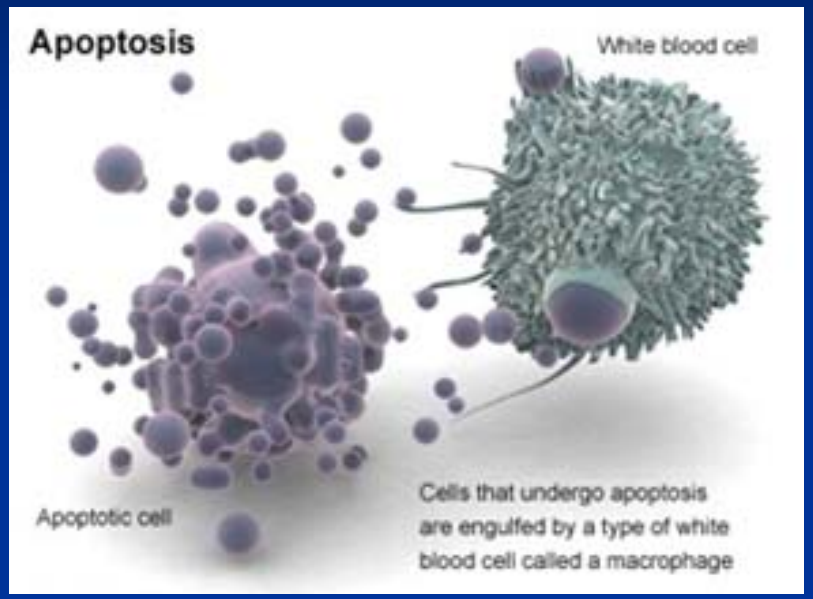


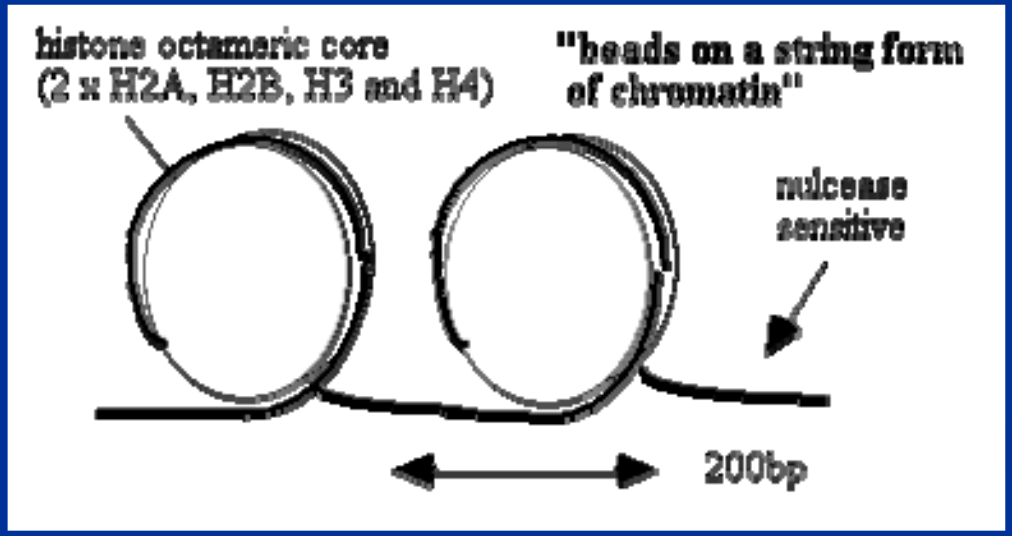
- **Bead is a nucleosome with about 160 bp of DNA wrapped twice around a core of 8 histones.**
- **40 bp of DNA link together nucleosomes.**

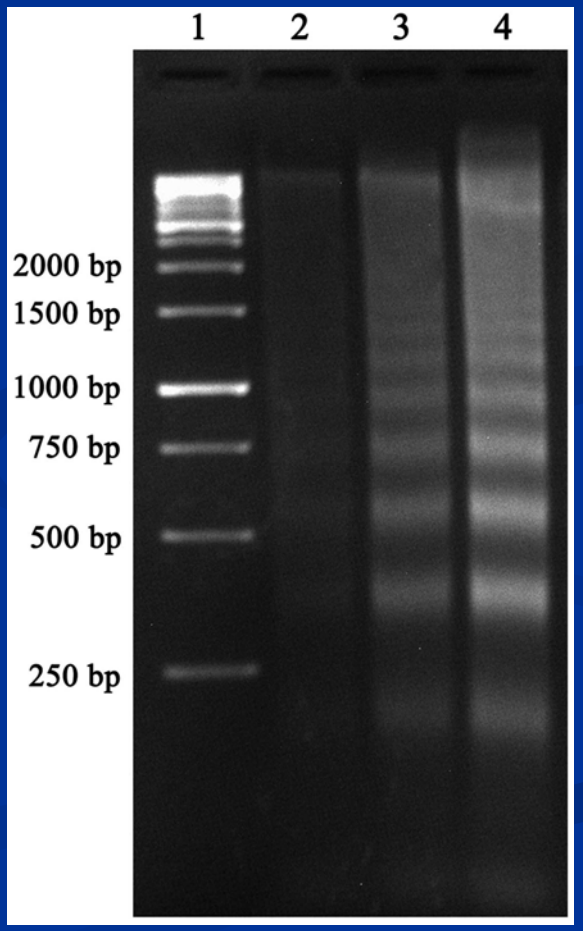
Fig. 11.4

The DNA laddering assay of apoptosis:

- DNA was fragmented into ~180 bp during apoptosis by the caspase-activated DNase (CAD).



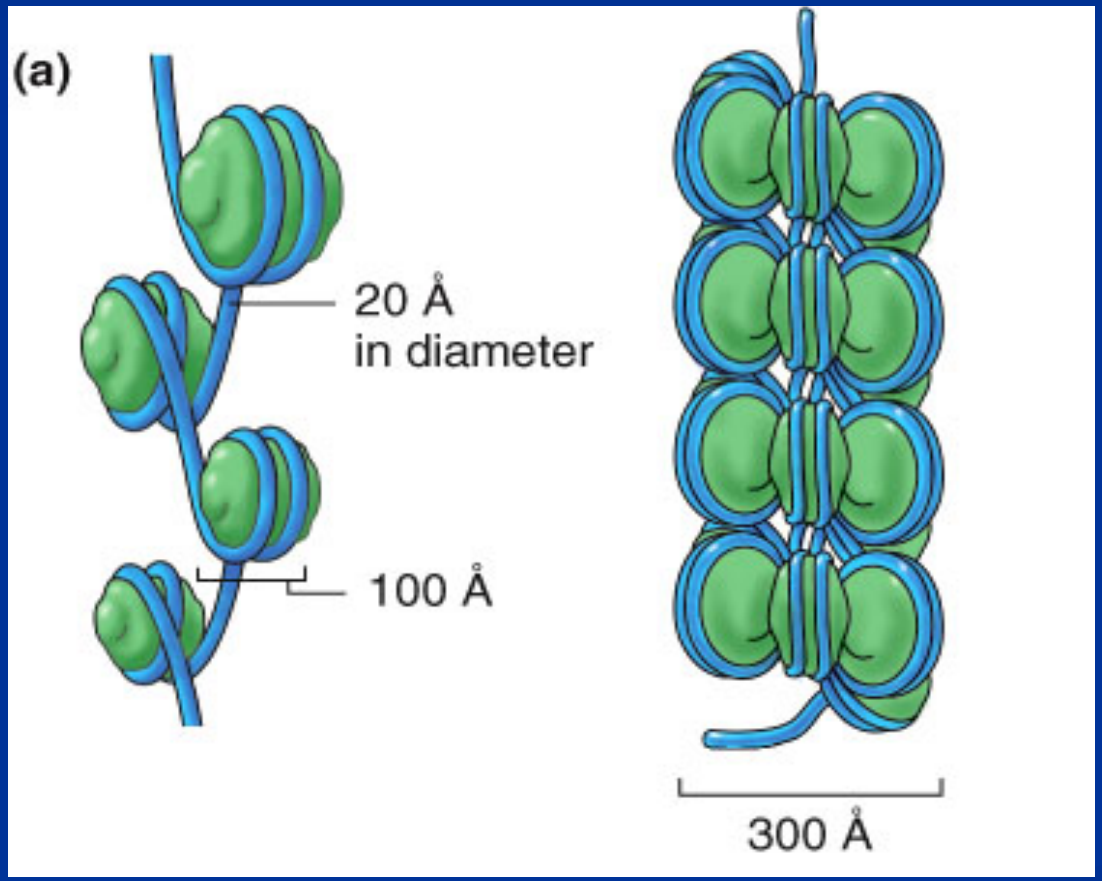


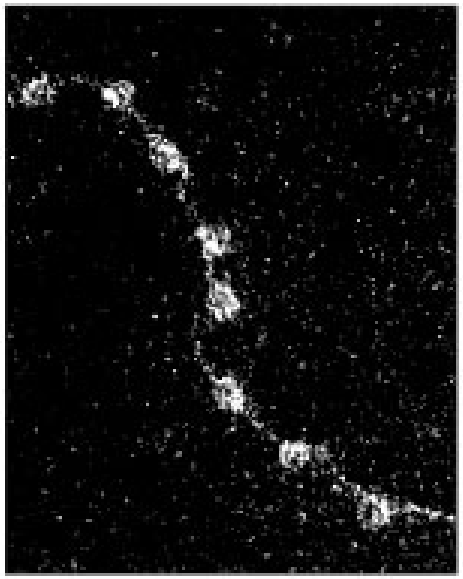


- **Spacing and structure of nucleosomes affects gene expression.**
  - **DNA in the regions between nucleosomes is available for interactions with proteins involved in expression, regulation, and further compaction.**
  - **The way in which DNA is wound around a nucleosome plays a role in determining how certain proteins interact with specific DNA sequences.**
- **Packaging into nucleosomes condenses DNA 7-fold.**
  - **2 meters of DNA shortens to less than 0.25 meters.**

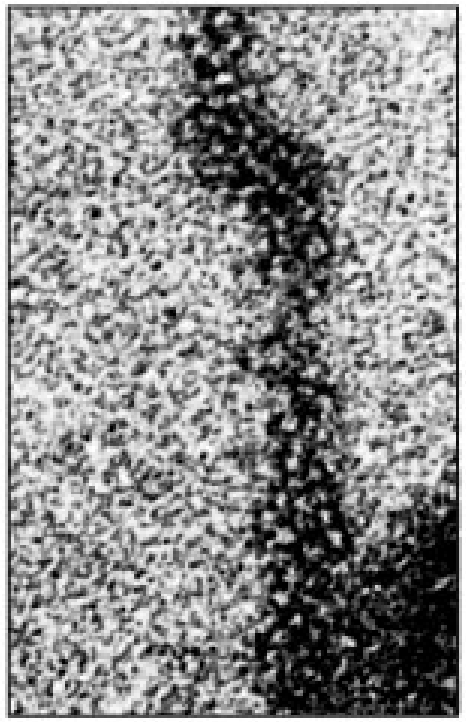
Models of higher level compaction seek to explain extreme compaction of chromosomes

- Formation of 300 Å fiber through supercoiling





100 Å fiber



300 Å fiber
(superhelix)

Fig. 11.5

- **Radial loop-scaffold model** for higher levels of compaction
  - Each loop contains 60-100 kb of DNA tethered by nonhistone scaffold proteins.

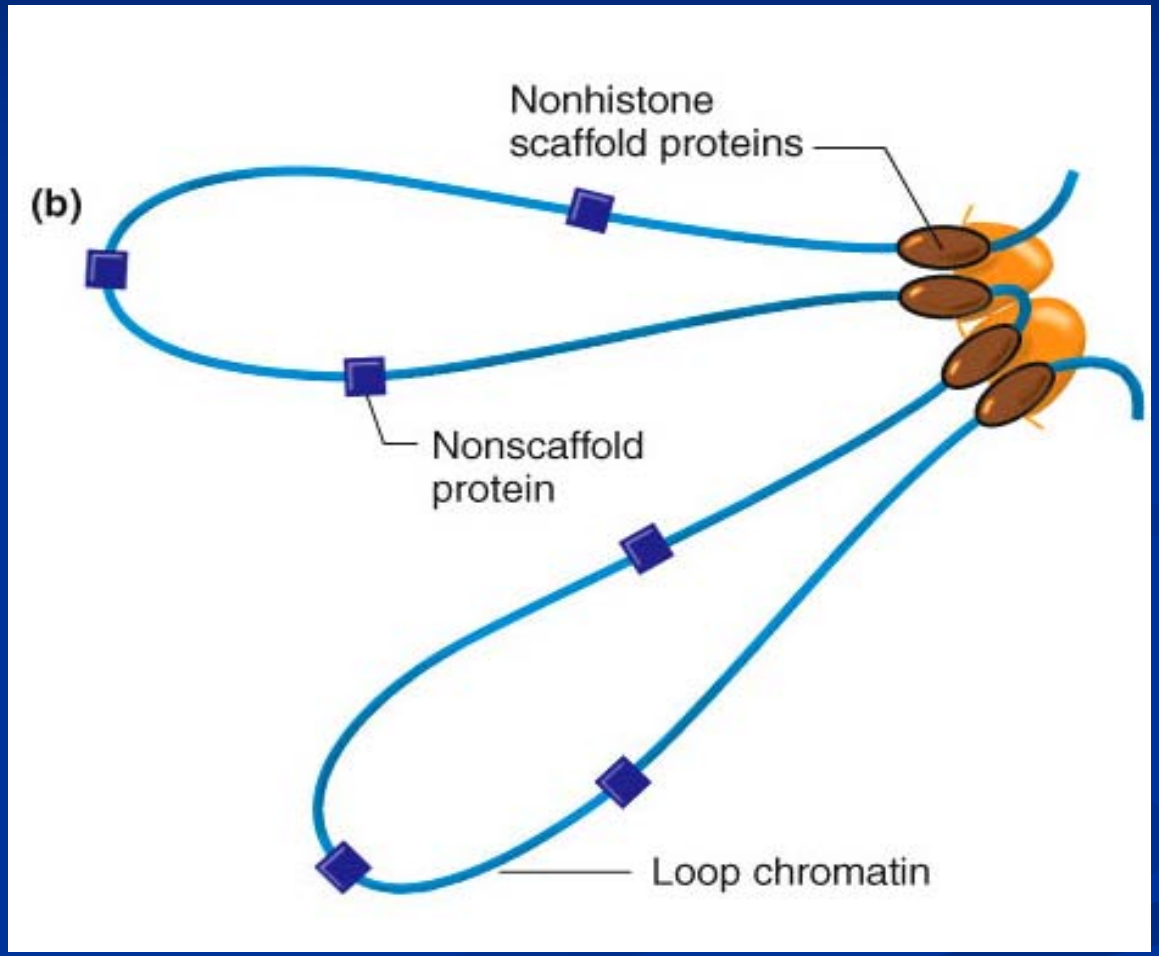


Fig. 11.5

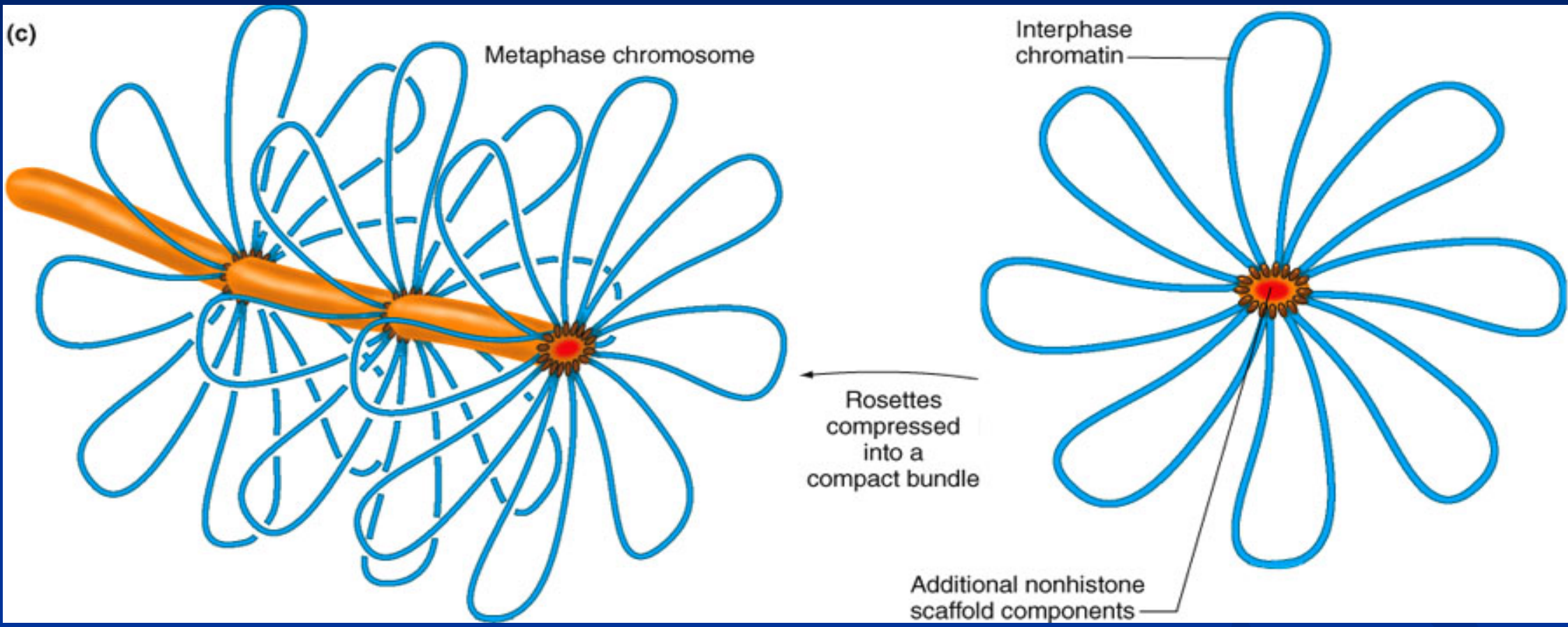
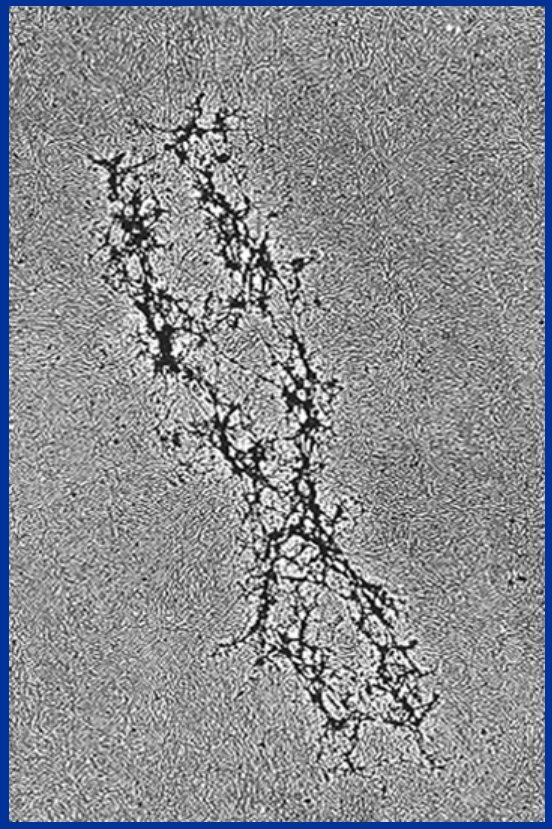

(c)
Metaphase chromosome
Interphase chromatin
Rosettes compressed into a compact bundle
Additional nonhistone scaffold components

Fig. 11.5

Experimental support for radial loop-scaffold model

- Electron micrograph shows long DNA loops emanating from the protein scaffold.



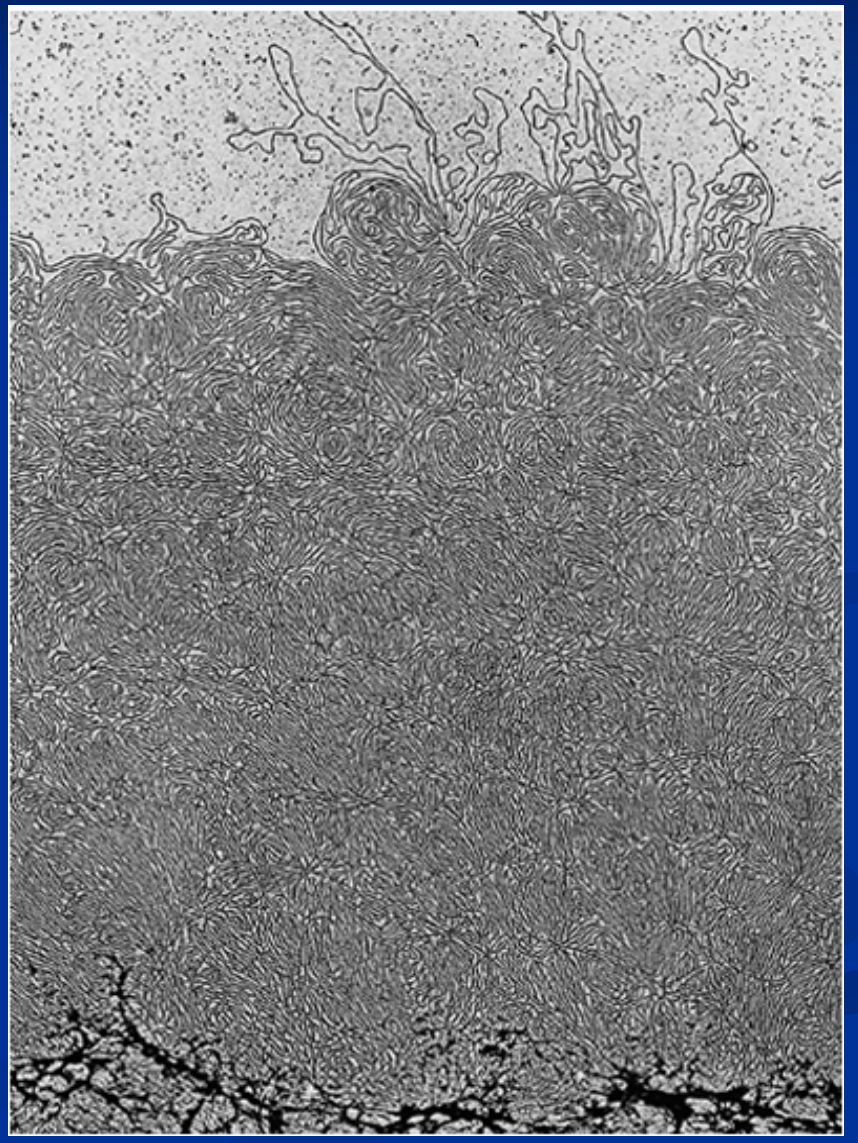


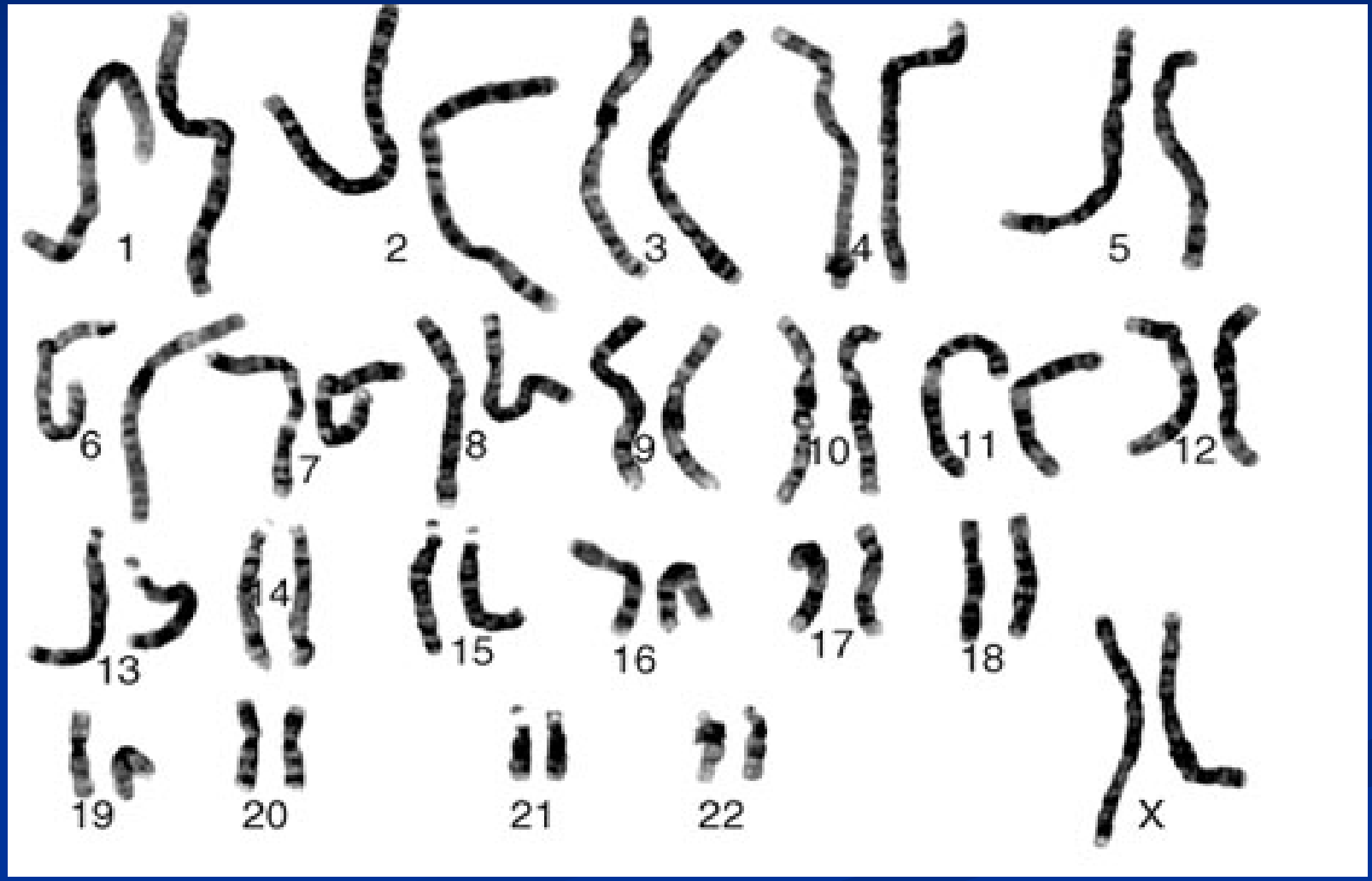
Fig. 11.1 and 11.6

TABLE 13.2 Different Levels of Chromosome Compaction

Mechanism	Status	What It Accomplishes
Nucleosome	Confirmed by crystal structure	Condenses naked DNA 7-fold to a 100 Å fiber
Supercoiling	Hypothetical model (although the 300 Å fiber predicted by the model has been seen in the electron microscope)	Causes additional 6-fold compaction, achieving a 40- to 50-fold condensation relative to naked DNA
Radial Loops—Scaffold	Hypothetical model (preliminary experimental support exists for this model)	Through progressive compaction of 300 Å fiber, condenses DNA to rodlike mitotic chromosome that is 10,000 times more compact than naked DNA

A closer look at karyotypes: fully compacted metaphase chromosomes have unique, reproducible banding patterns

- "G bands"
- Banding patterns are highly reproducible.
- Not known what they represent.



- ~ 300 dark and light G bands in low resolution human karyotype.
- ~ 1000 G bands in high-resolution human karyotype.
- **Banding patterns** help locate genes.

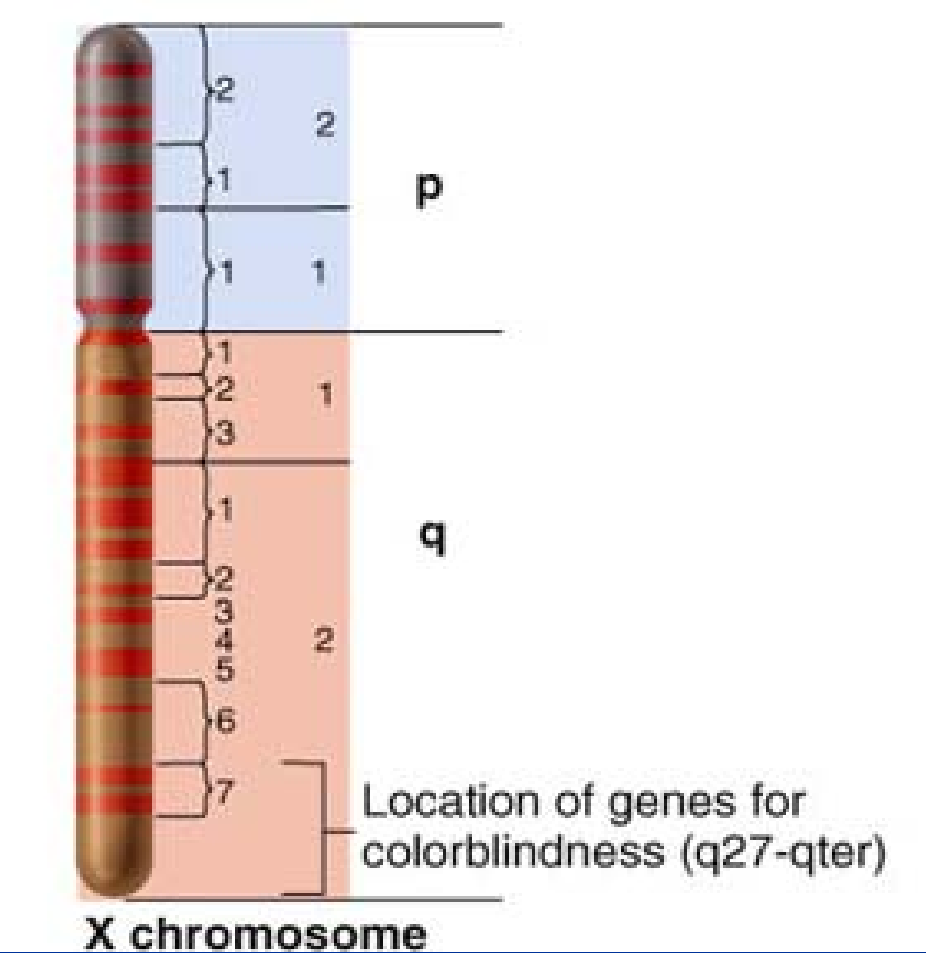
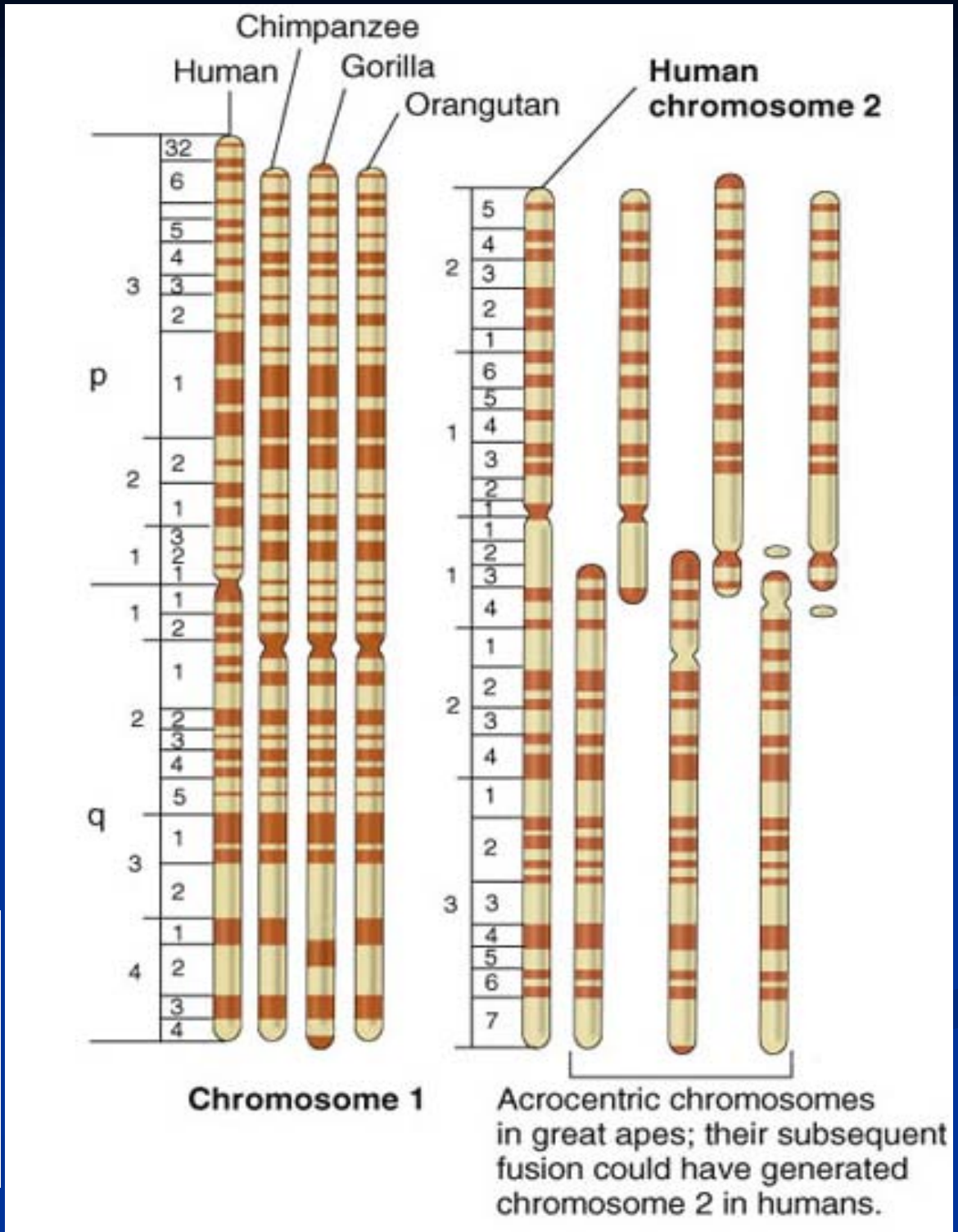


Fig. 11.8

- **Banding patterns** can be used to analyze chromosomal differences between species.
- Can also be used to reveal cause of genetic disease.
  - e.g., **Down syndrome** – 3 copies of chromosome 21.
  - Deletion of a region on X chromosome.



Fluorescent *in situ* hybridization (FISH) helps geneticists characterize genomes

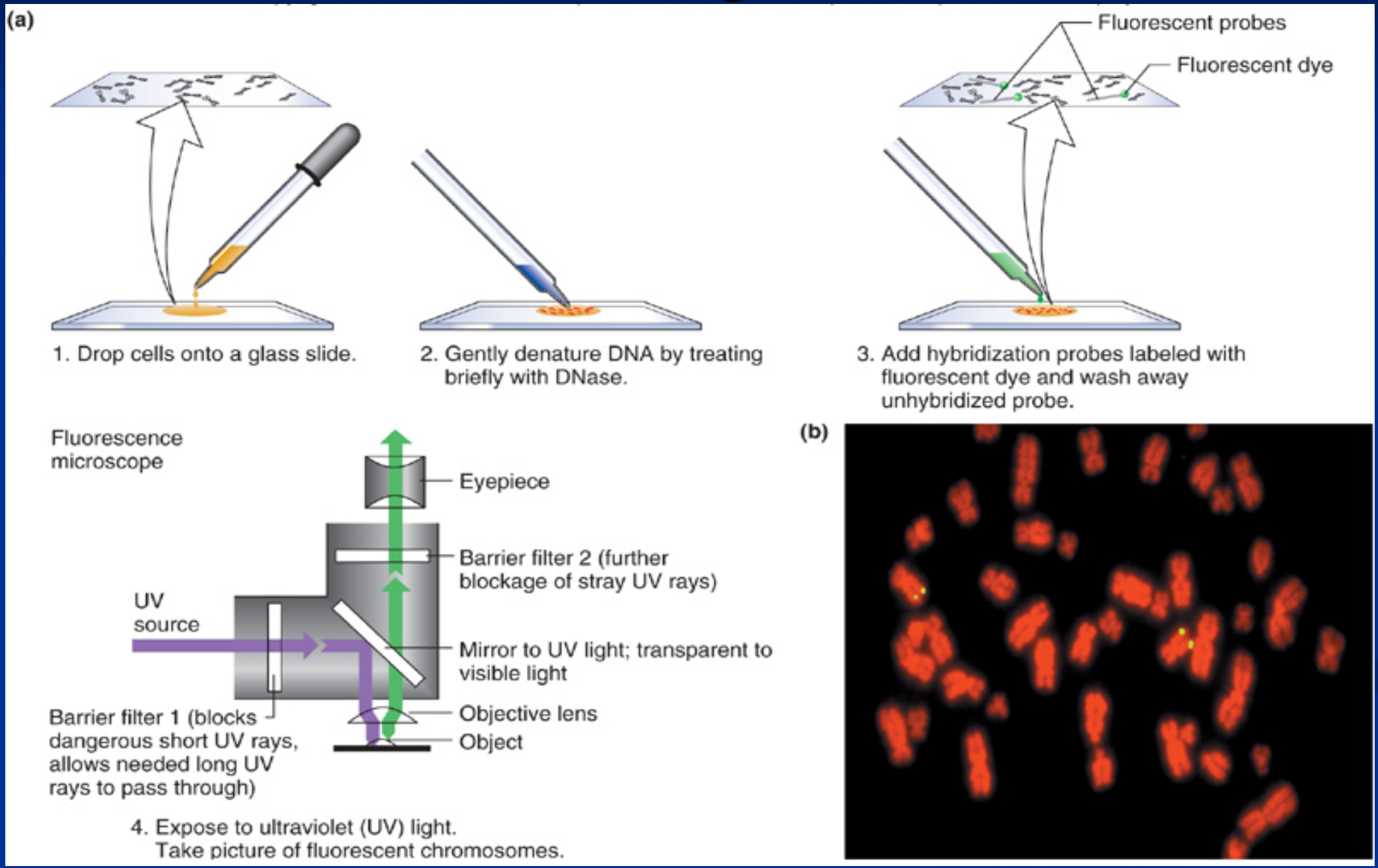


Fig. 11.9

11.3 Chromosomal packaging and gene expression

- Compaction of DNA into chromatin hinders DNA replication, DNA damage repair, and gene transcription.
- How can these functions be carried out?
  - Chromatin structure is dynamic and can change to allow the access of specific proteins.
  - Variations exist in the molecules making up the basic chromatin structure.

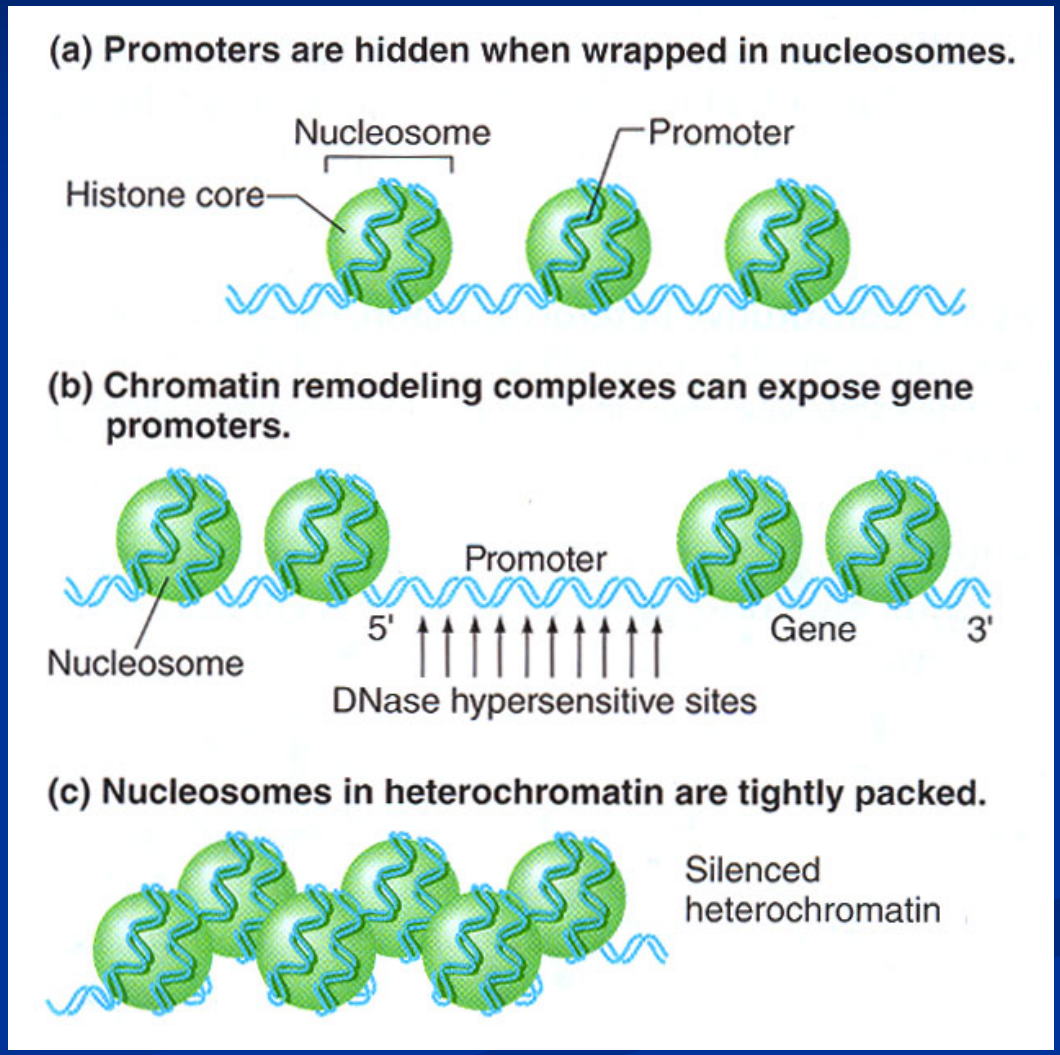
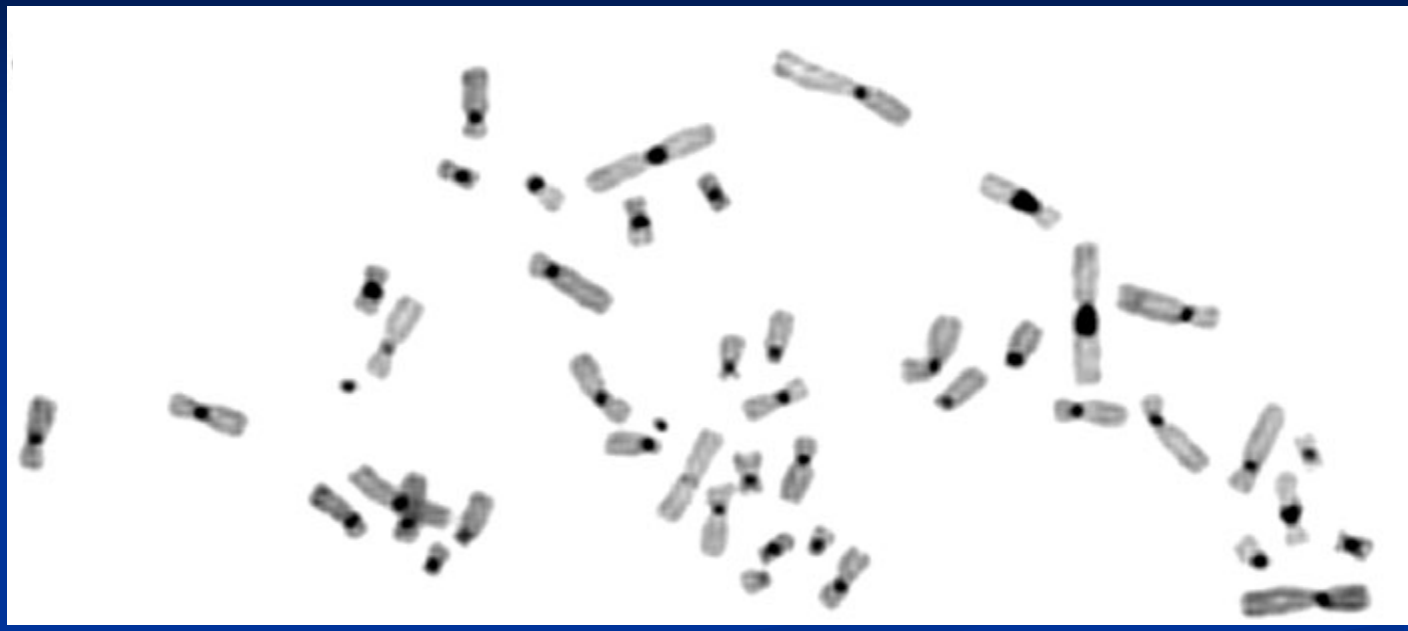


Fig. 11.10

Heterochromatin versus euchromatin

- Heterochromatin is darkly stained.
- Euchromatin is lightly stained.
- C-banding techniques stains constitutive heterochromatin near centromere in humans.



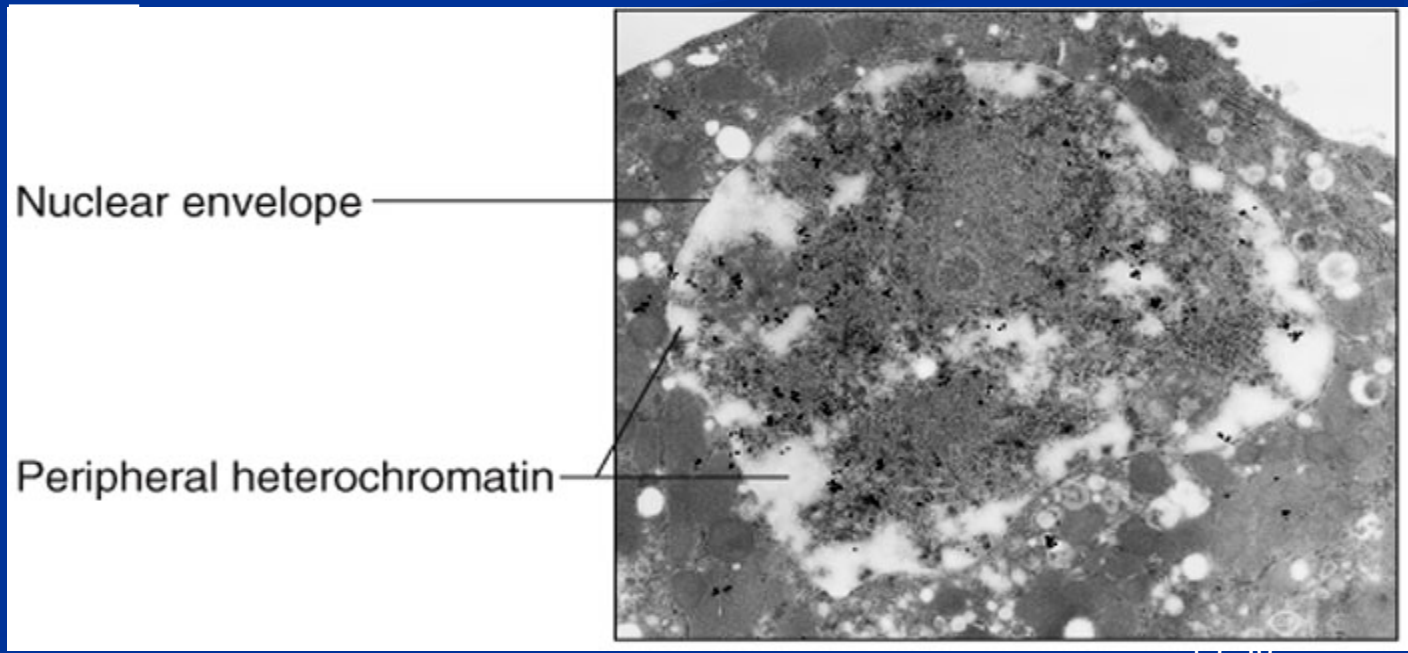


Fig. 11.11

Most genes in heterochromatin regions are silenced

- **Euchromatin**
  - Lightly stained regions of chromosomes
  - Is transcriptionally active, contains most genes.
- **Heterochromatin**
  - Darkly stained region of chromosome, usually found in regions near centromere.
  - Highly compacted even during interphase
  - **Constitutive heterochromatin:** remains condensed most of time in all cells, e.g. Y chromosome in fruitfly.

Heterochromatin formation correlates with the loss of gene activity

- **Position-effect variegation** in *Drosophila*
- **X chromosome inactivation** in female mammals

Heterochromatin can spread along a chromosome and silence nearby euchromatic genes

- **Position-effect variegation (PEV).**
- Moving a gene near **heterochromatin** silences its activity in some cells but not in others.
- First identified by **Hermann Muller** (1946 Nobel laureate) in 1938.

Hermann Muller
(1890-1967)

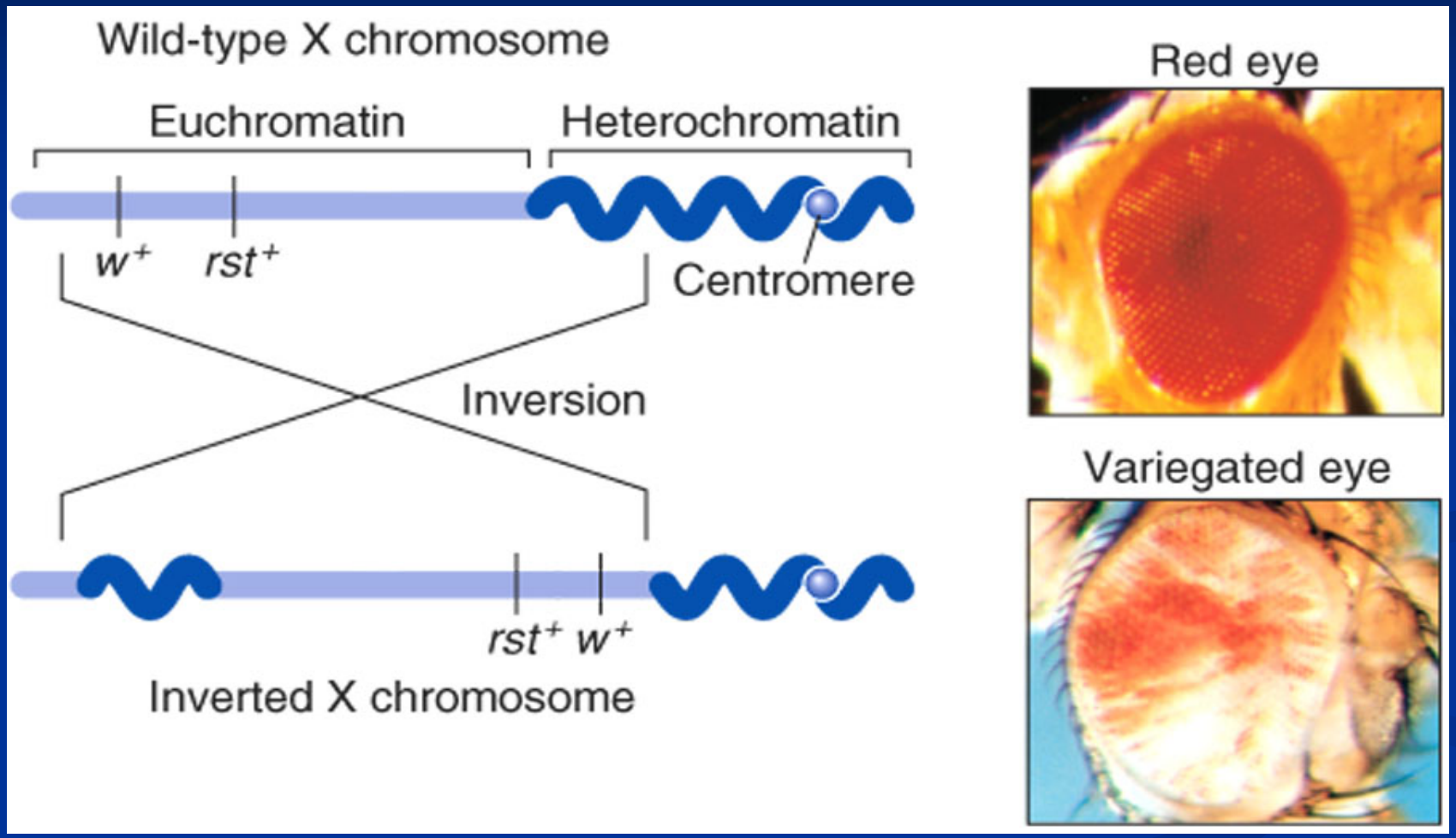

Wild-type X chromosome
Euchromatin
Heterochromatin
w+
rst+
Centromere
Inversion
rst+ w+
Inverted X chromosome
Red eye
Variegated eye

Fig. 11.12

A model for position-effect variegation

- Heterochromatin can spread to nearby genes and causes their inactivation.
- Heterochromatin can spread different distances in different cells, but it usually does not skip genes.

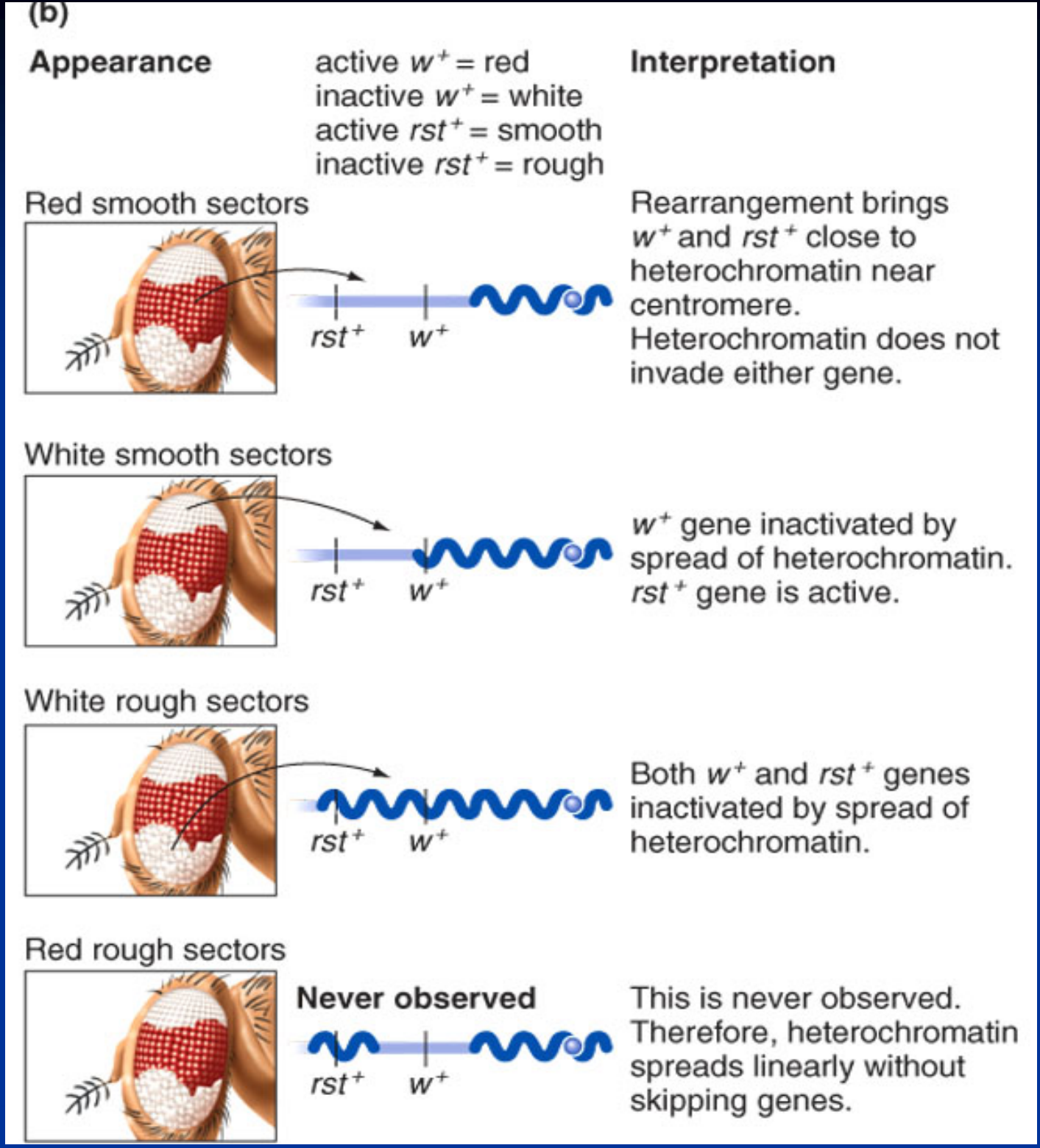


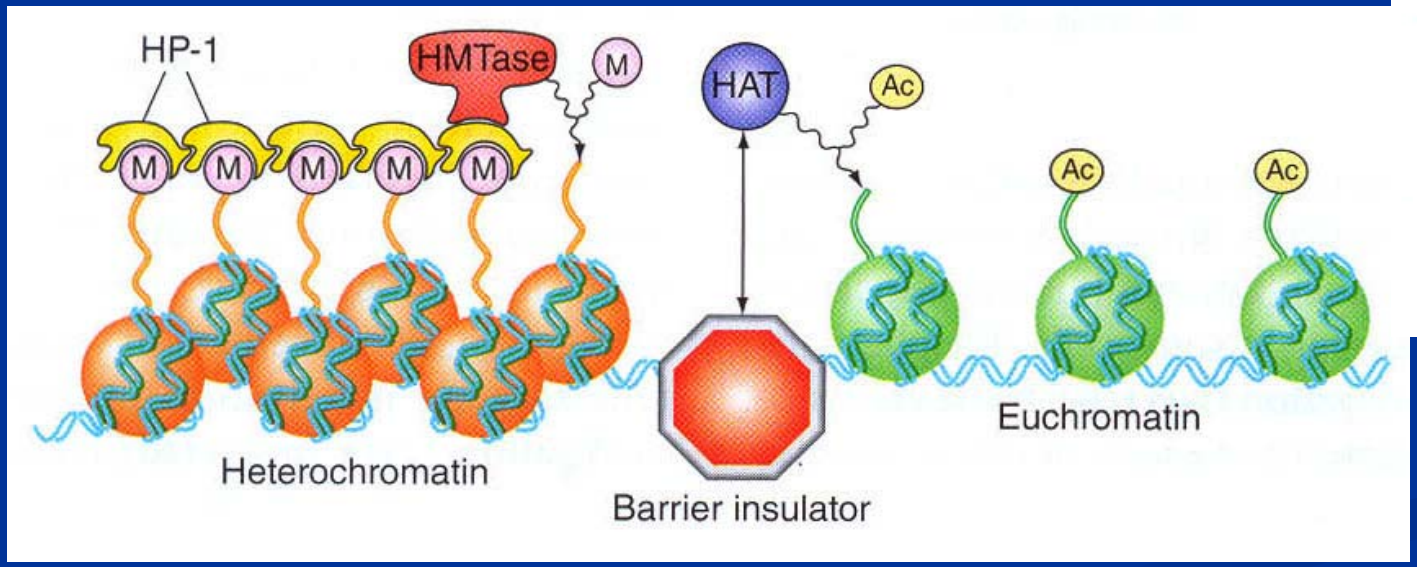
Fig. 11.12

Facultative heterochromatin (兼性异染色质):

Regions of chromosomes (or even whole chromosomes) that are heterochromatic in some cells and euchromatic in other cells of the same organism.

Heterochromatin and euchromatin have different histone modifications

- Histone tails can be modified: acetylation at specific lysines and methylation on specific lysines and arginines.



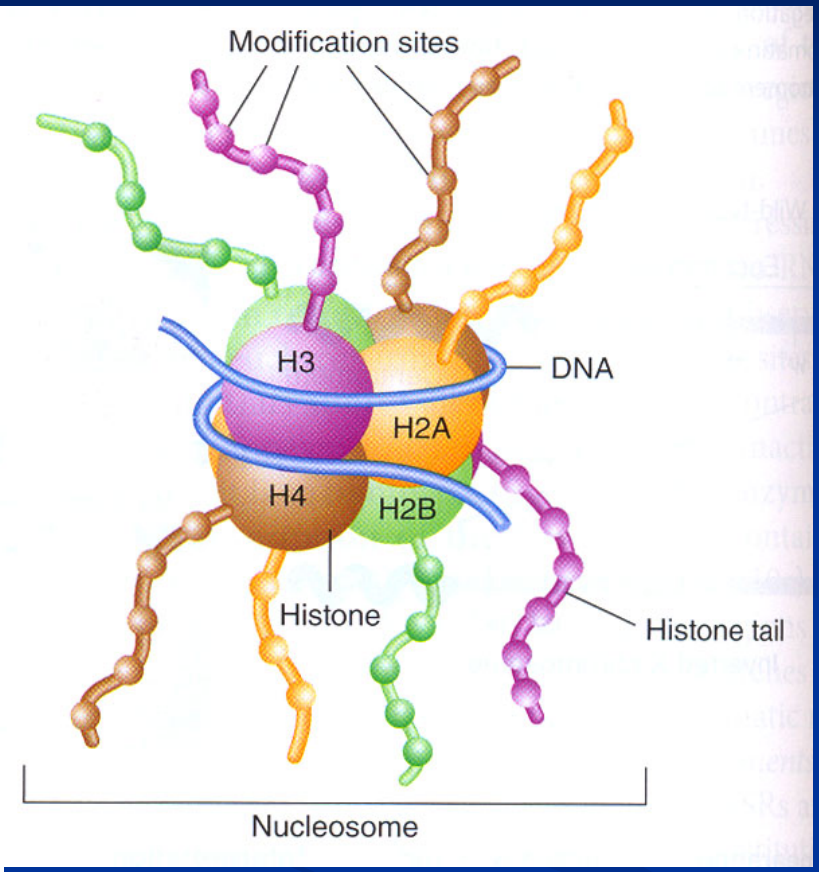
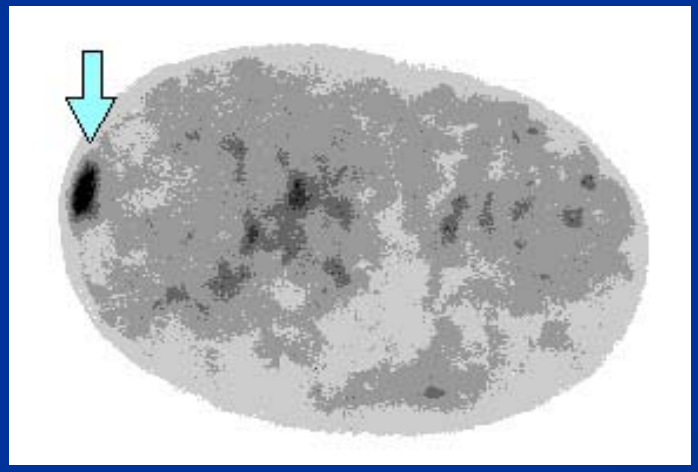


Fig. 11.13, 11.14

Heterochromatin formation inactivates an X chromosome in cells of female mammals

- In a female mammal, one X chromosome in the interphase cells appears as a darkly stained heterochromatin mass, called **Barr bodies**.
- Barr bodies were discovered by **Murray Barr**, a medical researcher in 1948.

Barr body



Murray Barr (1908-1995)

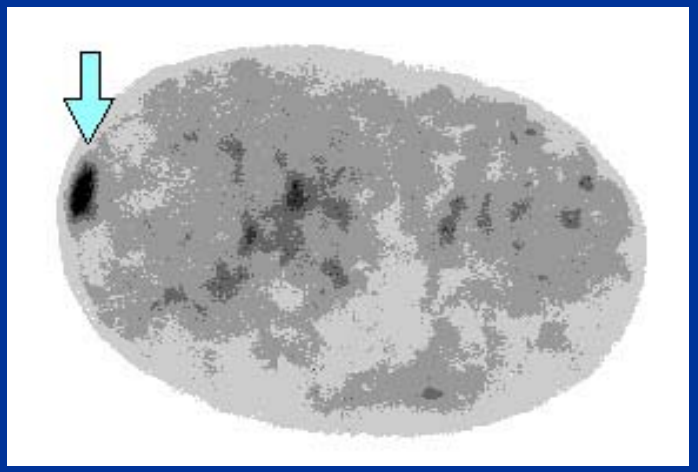
- In 1961, Mary F. Lyon proposed that in female mammals all X chromosomes but one were inactivated.

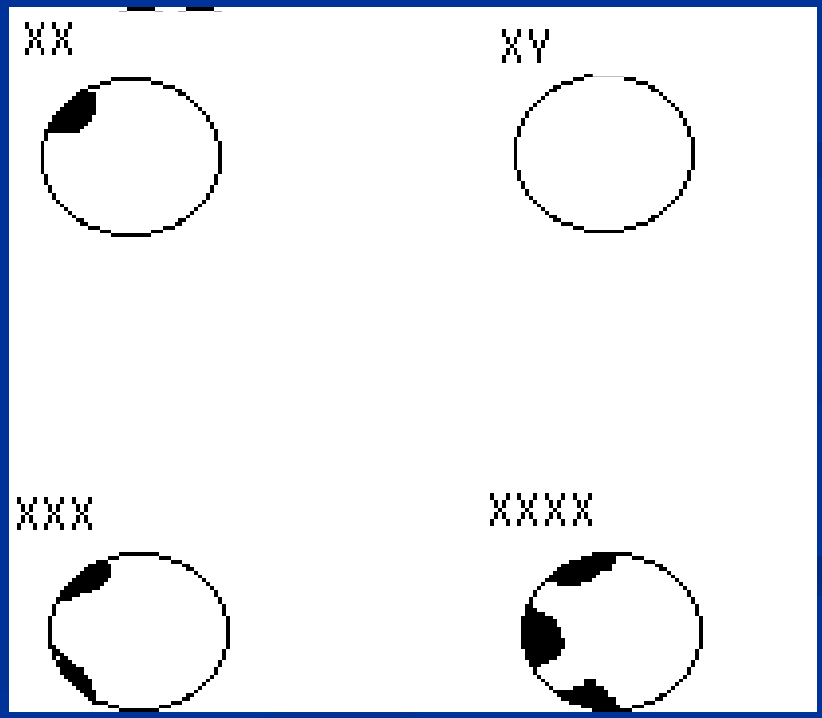
Mary F. Lyon
(1925-)

The Lyon hypothesis:

1. Each cell has only one active X chromosome. All the other ones are inactivated.
2. The inactivation occurs in early embryonic development.
3. In a particular cell, which X chromosome will be inactivated is randomly determined.

Barr body





Glucose-6-phosphate dehydrogenase (G6PD)

Beutler E *et al.* (1962) The normal human female is a mosaic of X-chromosome activity: studies using the gene for G6PD-deficiency as a marker. *P.N.A.S.*

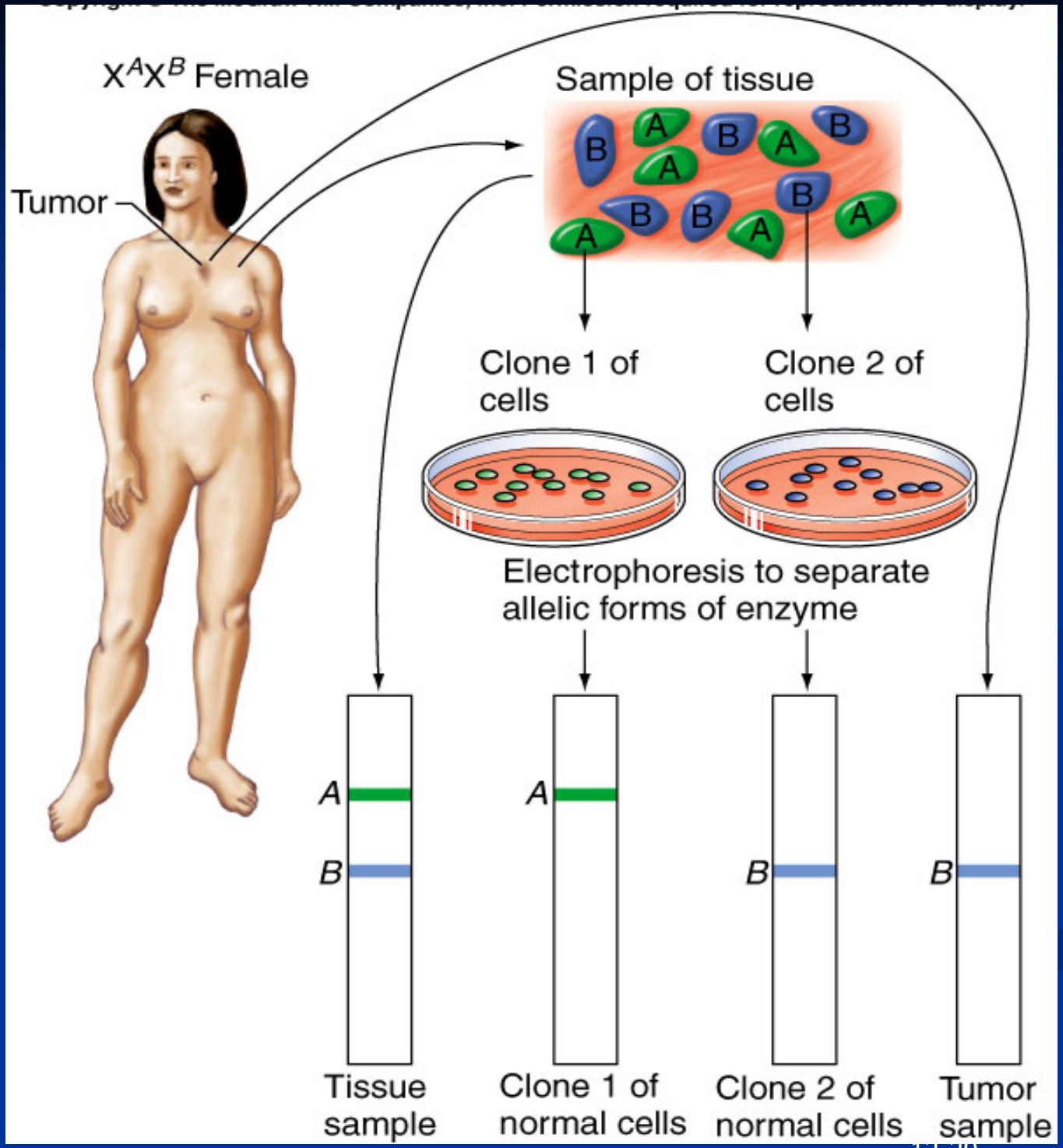
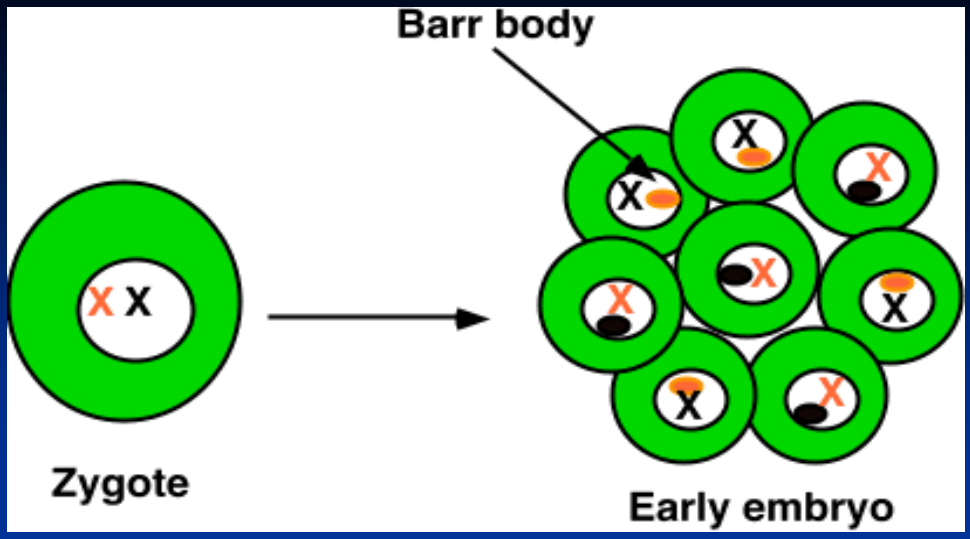
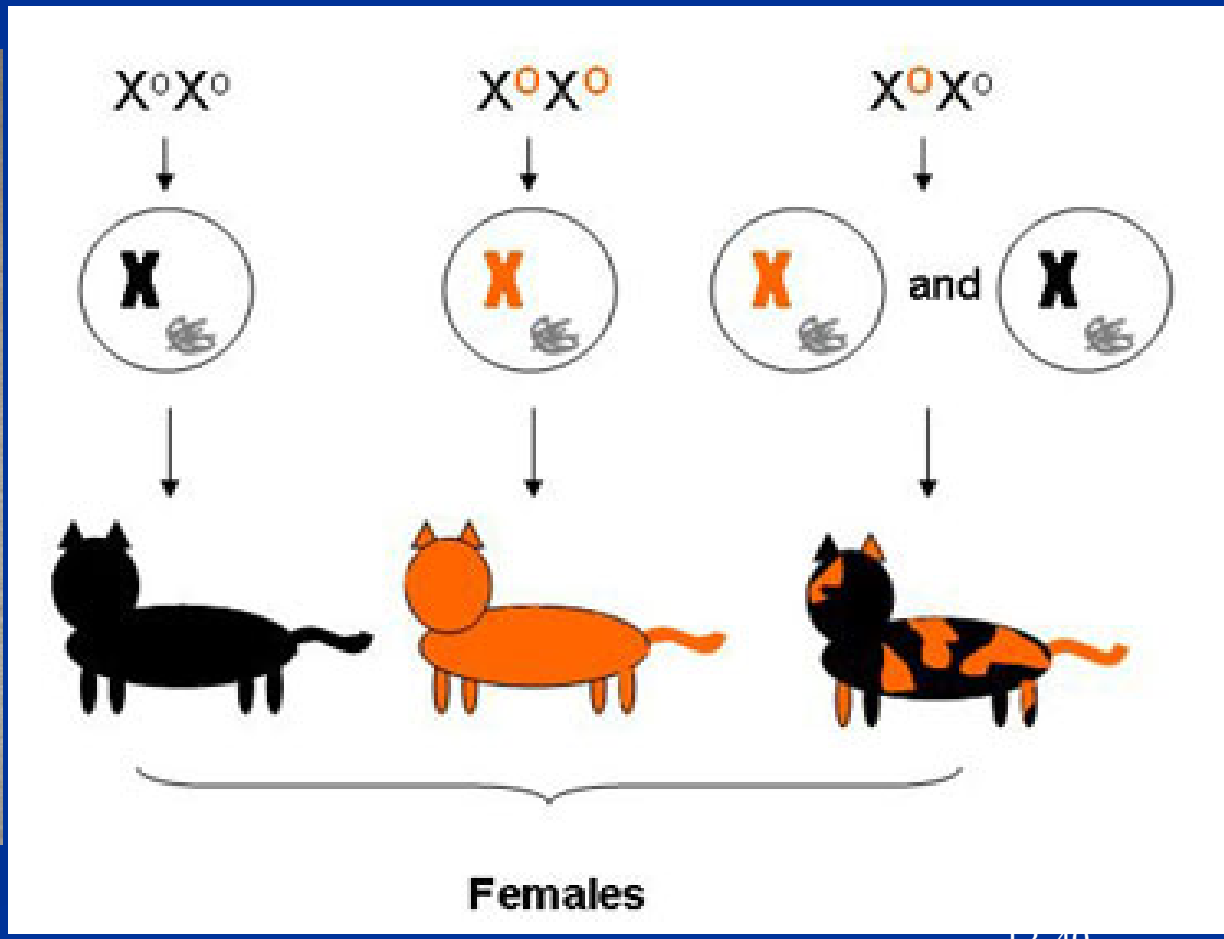


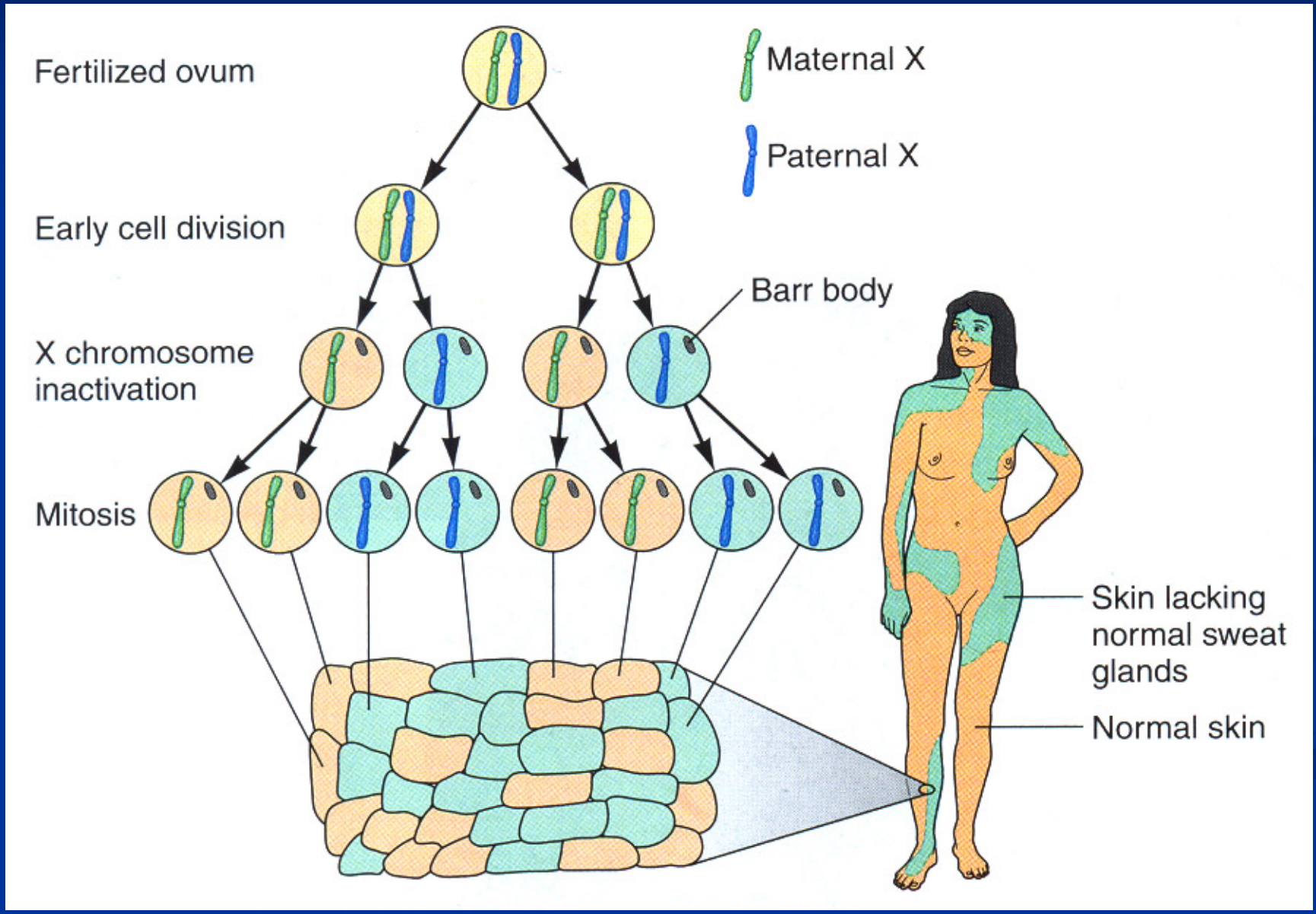
Fig. 19.7



Tortoiseshell cat



A female is a mosaic for expression of genes on the X chromosome

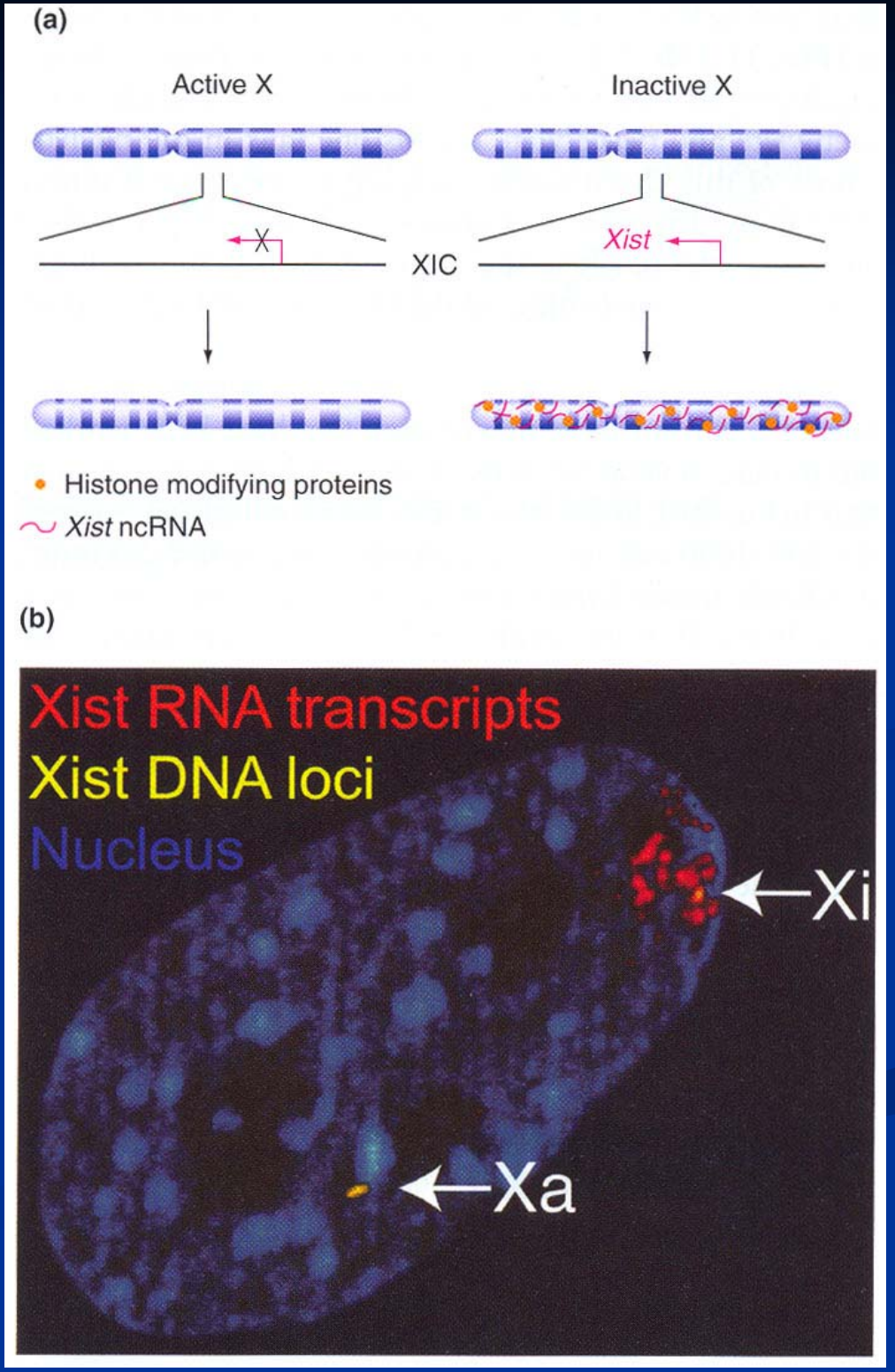


X chromosome inactivation exposed the effect of harmful disease-causing mutations

- "Mottled" feature of more than 16 human X-linked disorders in female heterozygotes involving the eye (retinitis pigmentosa) and skin (anhidrotic ectodermal dysplasia).
- Female carriers of Duchenne muscular dystrophy (DMD) are usually asymptomatic. However, 2.5-7.8% of them may present muscle symptoms and cardiomyopathy, attributed to a reduced production of dystrophin.

Mechanism of X chromosome inactivation

- A 450 kb region called X inactivation center (XIC) in human X chromosome.
- Xist, a ~17 kb noncoding RNA, was transcribed stably only from the inactive X chromosome.
- Xist ncRNA binds to many sites on the inactive chromosome and then attracts histone modifying enzymes that silence the DNA.

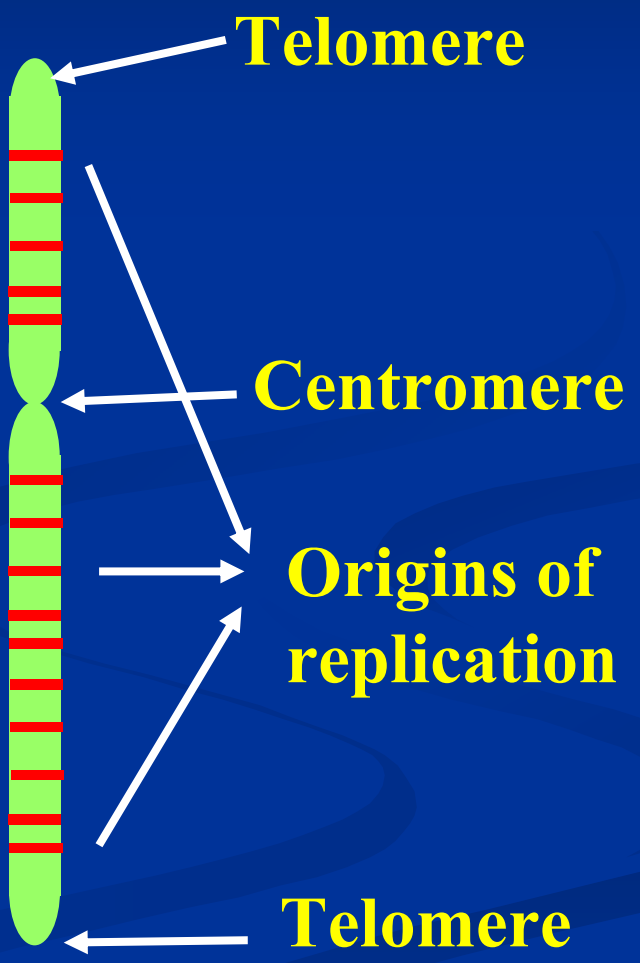


The nucleus of a XX female mouse cell

Fig. 11.15

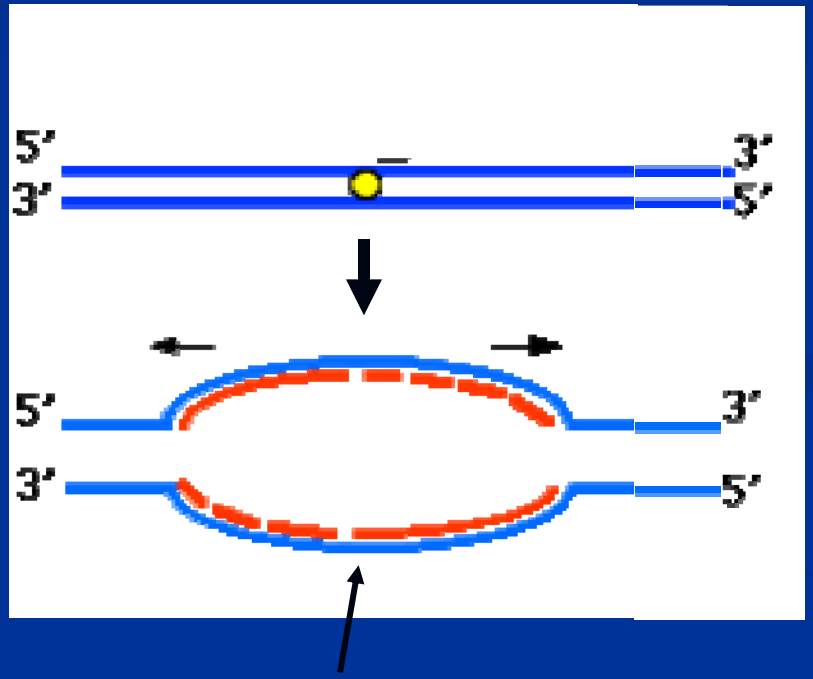
11.4 Replication of eukaryotic chromosomes

- Origins of replication
- Telomeres
- Centromeres

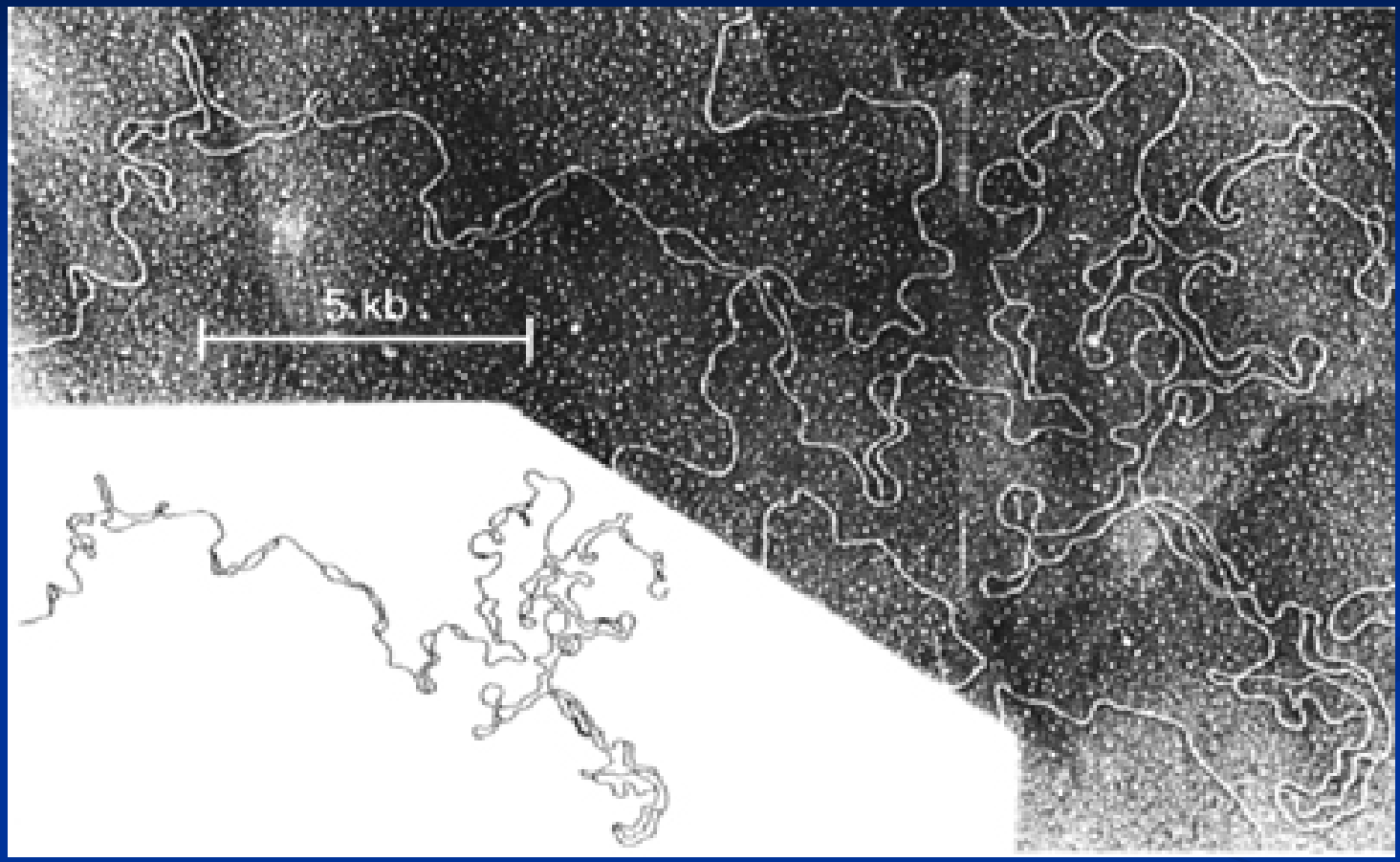


1. Origin of replication

Origin of replication **determines where DNA replication starts.**



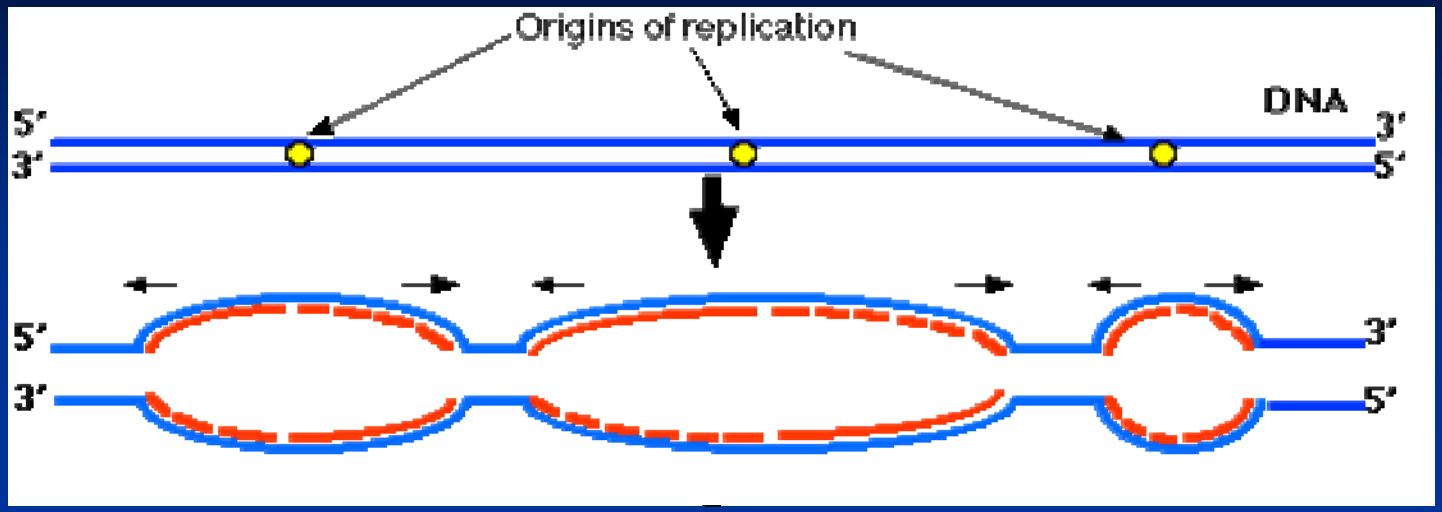
Replication bubble



Electron micrograph of replicating DNA from a *Drosophila* embryo

Fig. 11.16

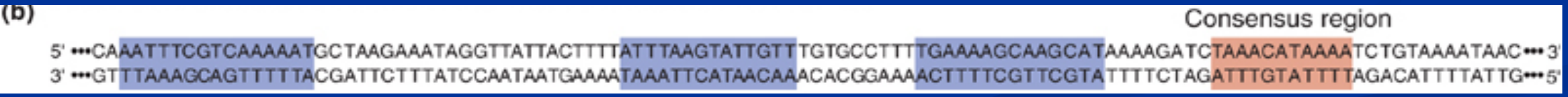
There are many origins of replication in eukaryotes



- 10,000 origins of replication in mammals, separated by 30 – 300 kb .
- Each bidirectional replication is called a **replicon**.
- DNA polymerase can assemble new DNA at a rate of about 50 nucleotides per second.
- Replication occurs in about 8 hours during S phase in actively dividing human cells.

Yeast origin of replication

- ***Autonomously replicating sequences*** **(ARSs) in yeast consist of an A – T rich region.**
- **ARSs permit replication of plasmids in yeast cells.**



Yeast ARS1 sequence

- The “new” nucleosomes are a mixture of old (recycled) and newly formed histones, distributed randomly on the two daughter DNA molecules.
- Synthesis and transport of histones must be tightly coordinated with DNA synthesis.
- Histone modifications in parental DNA become lost during DNA replication.

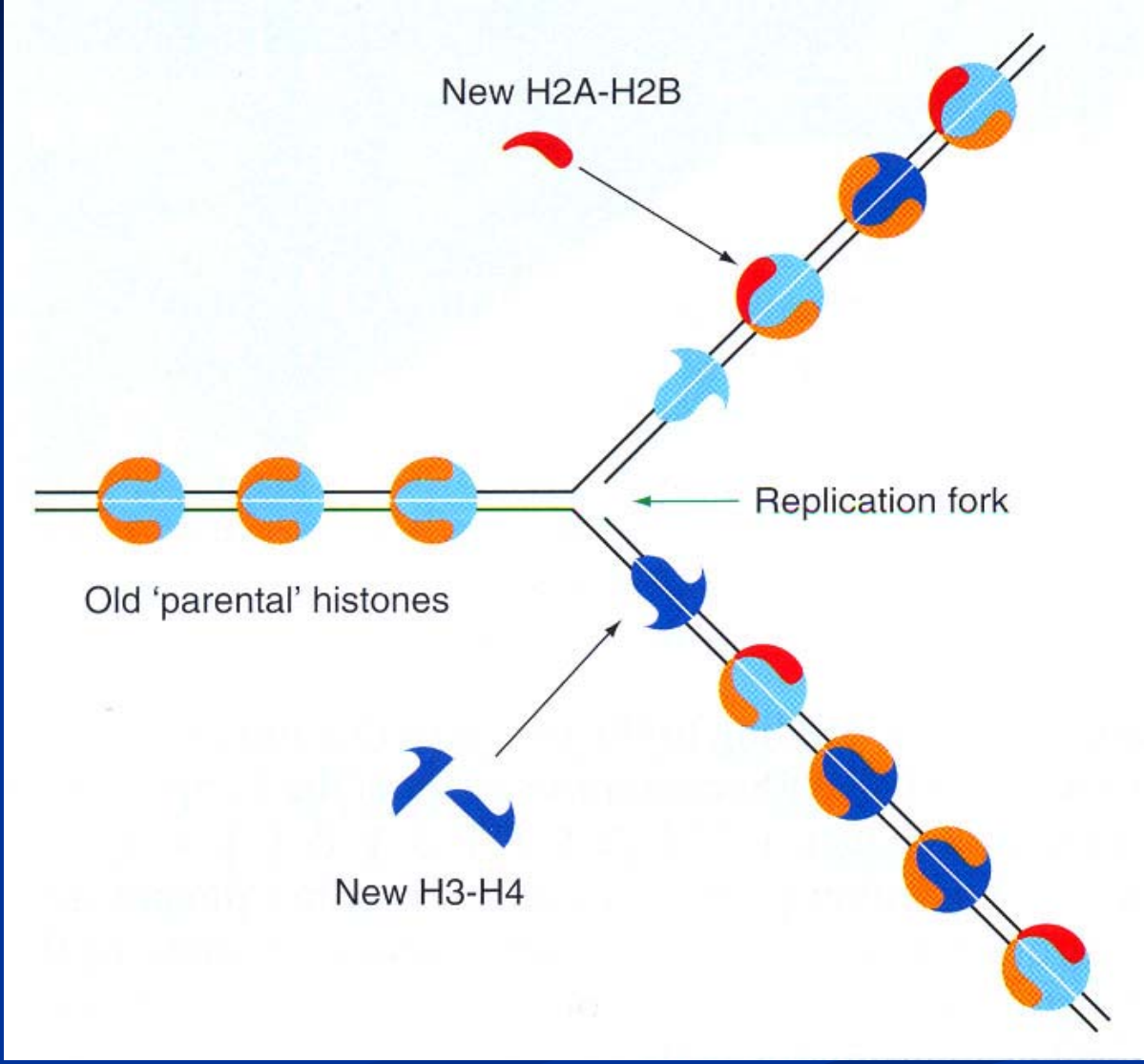


Fig. 11.17

2. Telomeres

Telomeres: Specialized terminal structures on eukaryotic chromosomes that ensure the maintenance and accurate replication of the two ends of each linear chromosome.

- **Telomeres are protective caps on eukaryotic chromosomes.**
  - **Prevent fusion with other chromosomes**
- **250-1500 TTAGGG repeats in yeast and humans.**

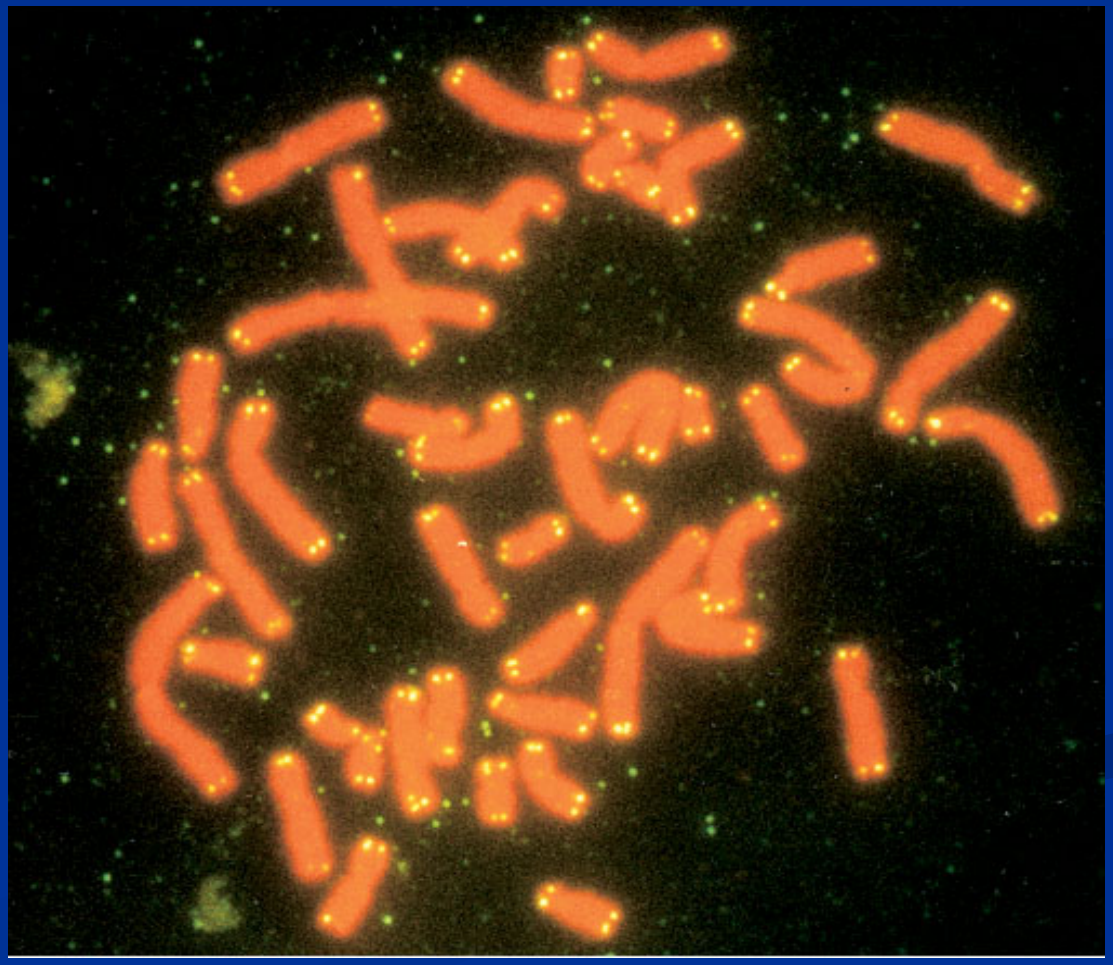


Fig. 11.18

Problem of end shortening for linear DNA

- **DNA polymerase cannot reconstruct 5’ end of a DNA strand.**

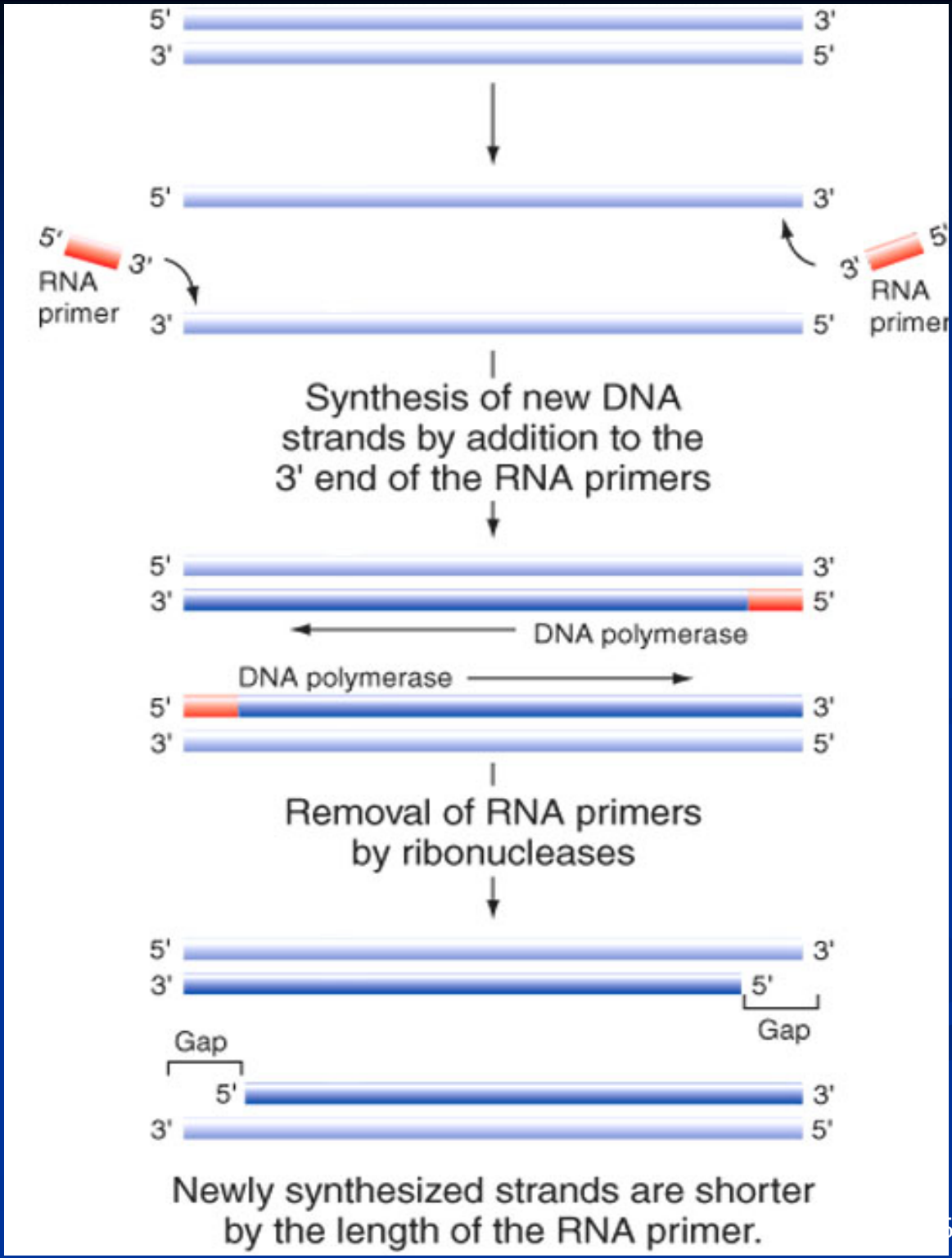


Fig. 11.19

Binding of telomerase to TTAGGG and addition of RNA extends the ends

Telomerase

- A reverse transcriptase that contains protein and RNA.
- RNA contains 3'AAUCCC5' repeats.

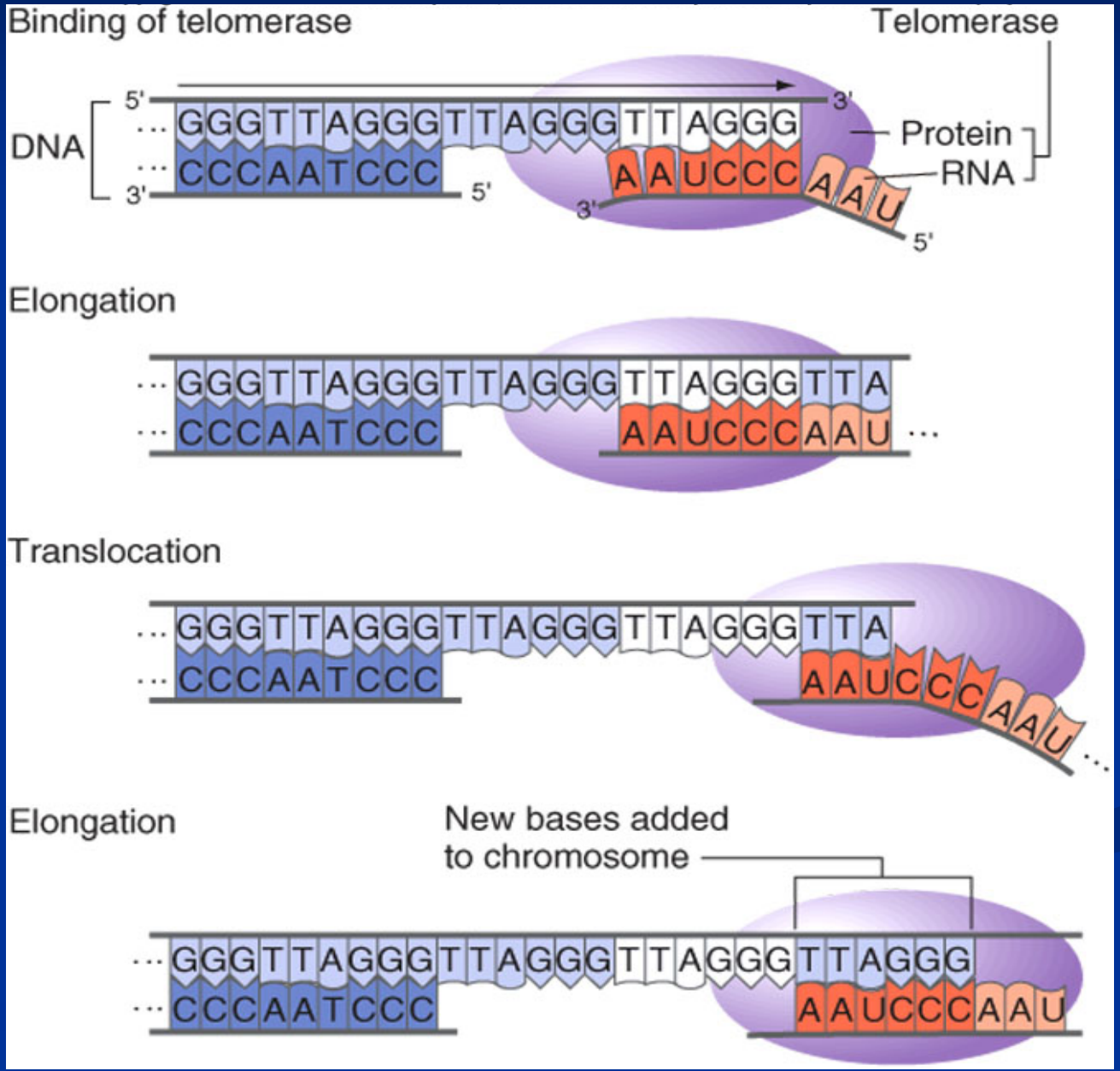


Fig. 11.20

Figure 11.21 The shelterin complex protects telomeres. The proteins of the shelterin complex (*colored shapes*) bind to telomeres, folding the DNA ends (*gray*) so they can neither be attacked by nucleases nor subjected to nonhomologous end-joining.

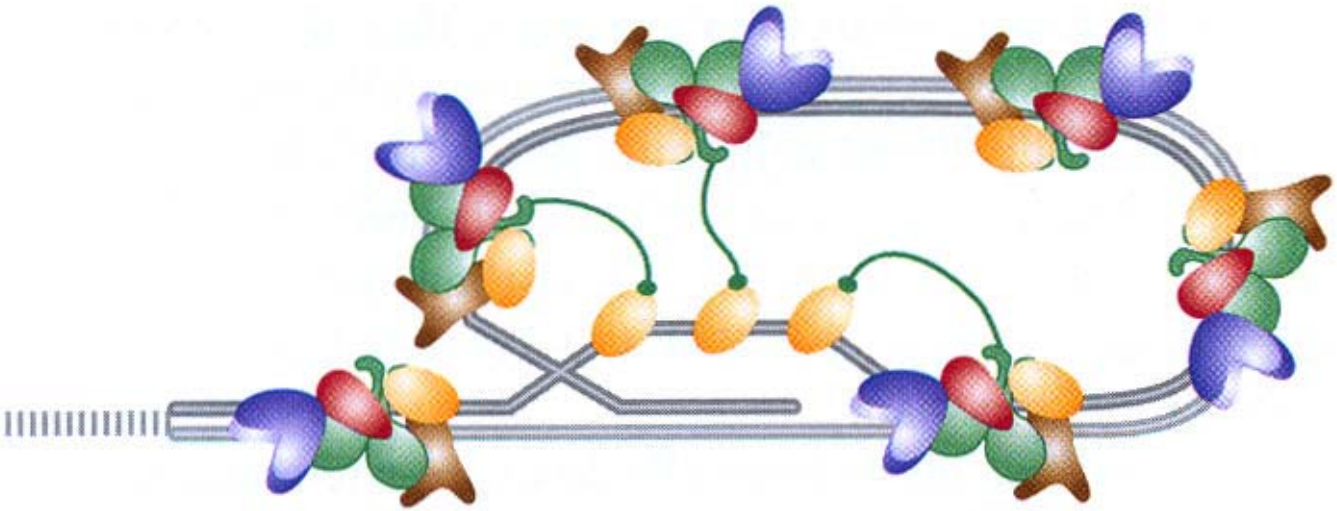


Fig. 11.21

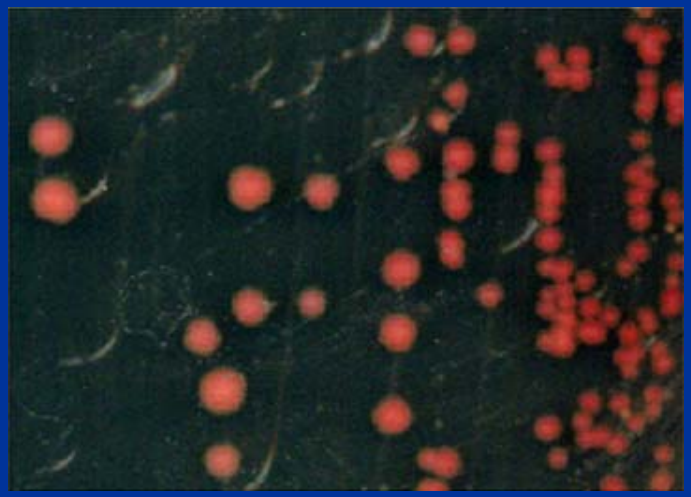
Yeast genetics helped the discovery of telomerase genes

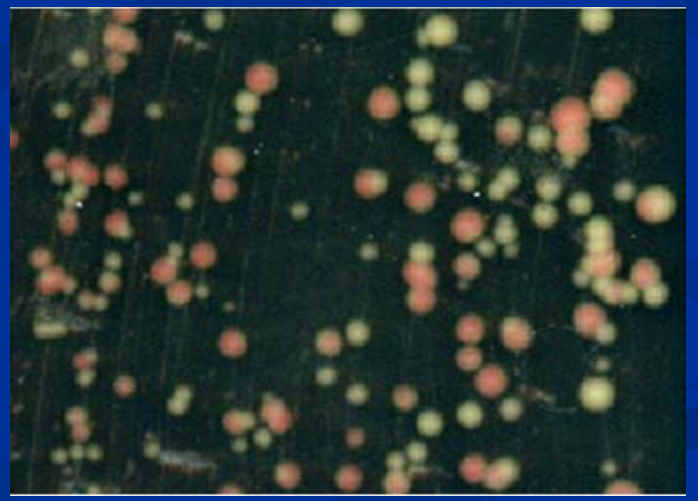
- ***TLC1*: RNA subunit**
  - **Identified by high-copy suppression of telomere position effect (TPE) (Singer MS and Gottschling DE 1994, *Science*).**

Daniel Gottschling
(Univ of Washington)

Ura⁻/5-FOA resistant
Ade⁻/ Red color

Ura⁺/5-FOA sensitive
Ade⁺/ White color

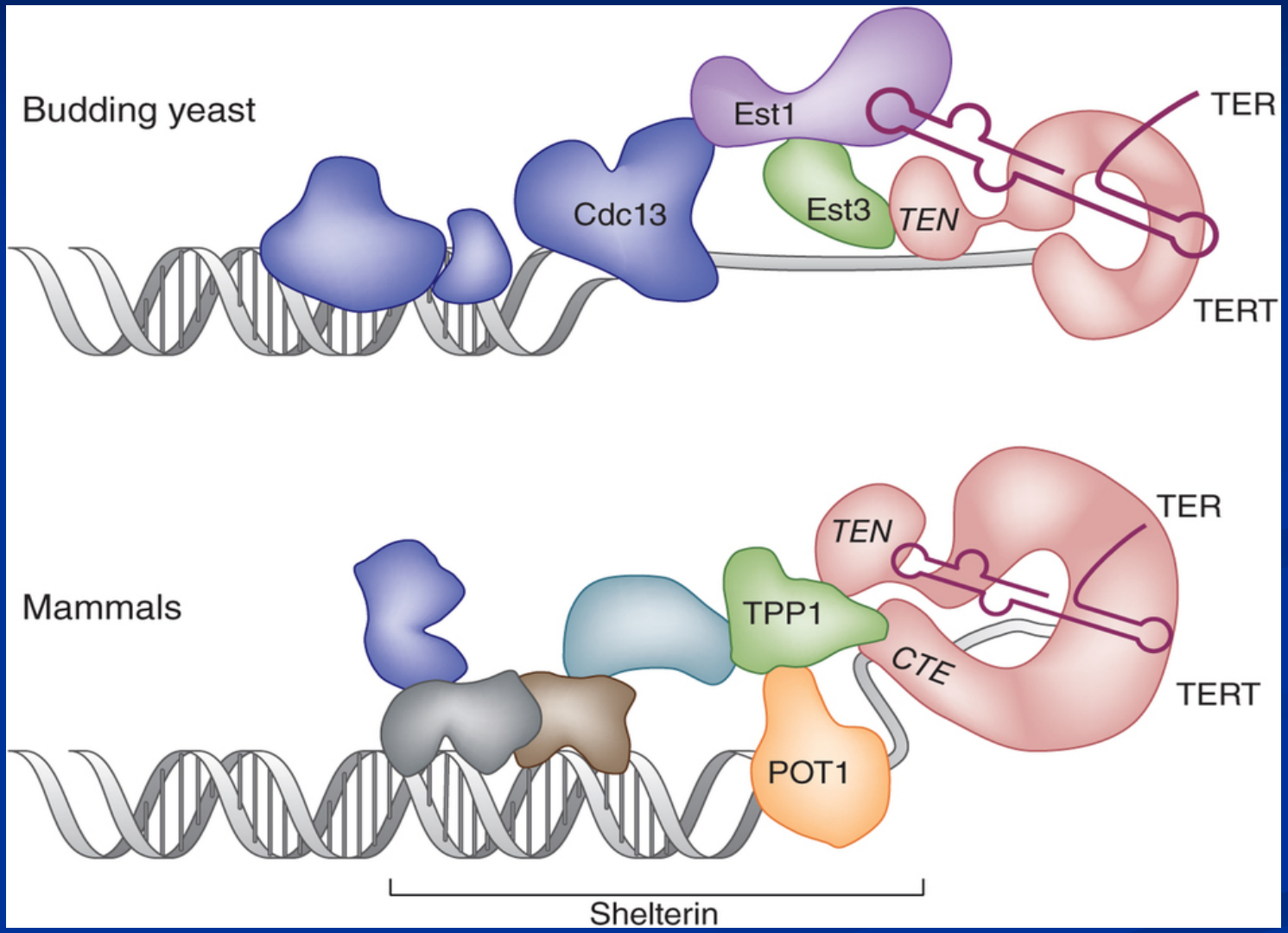




- ***EST1***, ***EST2*** **(reverse transcriptase),** ***EST3*****, and** ***CDC13*** **(*EST4*).**
  - **Identified by screening for mutants that showed a senescence phenotype and progressive shortening of telomeres (Lendvay TS *et al.* 1996 *Genetics*).**

Vicki Lundblad
(Salk Institute)

Telomerase

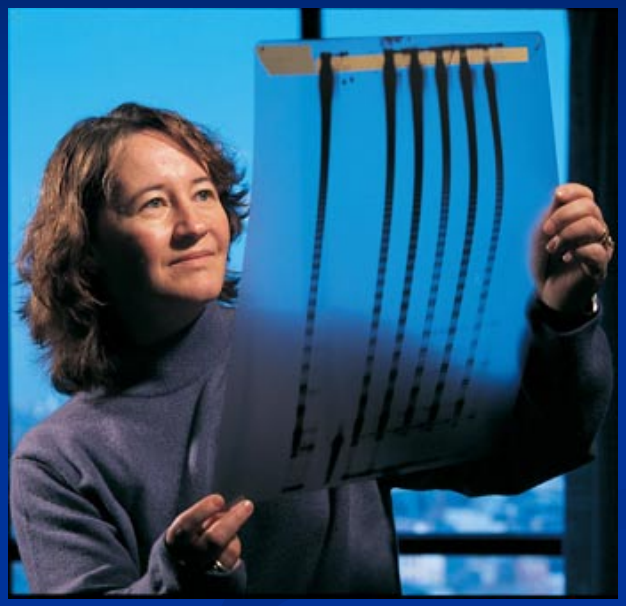

Budding yeast
Est1
TER
Cdc13
Est3
TEN
TERT
Mammals
TEN
TER
TPP1
CTE
TERT
POT1
Shelterin

Telomeres, aging, and cancer

- Yeast cells deleted the gene for telomerase undergo telomere shortening at the rate of about 3 bp per generation and died later.
- Telomerase may play a role in aging.
  - Most human somatic cells have very low levels of telomerase, and die after 30-50 rounds of cell division.
  - Germ-line cells and stems cells express relatively higher level of telomerase and can divide many more generations.
  - In 90% of cancers, abnormal overproduction of telomerase permits unlimited cell proliferation.

The 2009 Nobel Prize in Medicine or Physiology

Elizabeth Blackburn
UCSF



Carol Greider
Johns Hopkins U

Jack Szostak
Harvard U

Telomerase-independent mechanisms to achieve telomere elongation

- Some organisms do not naturally contain telomerase.
  - Some Dipteran insects including the fruitfly *Drosophila melanogaster*.
- Some organisms can live when telomerase is inactivated.
  - Survivors of some budding yeast *S. cerevisiae* mutants deleted the gene for telomerase.
  - Some immortalized mammalian cell lines and tumors.

Alternative telomere elongation mechanisms

- **Transposable element-based telomere elongation**
  - **Transposition of telomere-specific retrotransposons HeT-A and TART in the fruitfly *D. melanogaster*.**
- **Telomere-telomere recombination**
  - ***RAD52*-dependent break-induced recombination between chromosomal ends in the yeast *S. cerevisiae*.**
- **Circularization of linear chromosomes**
  - **In the yeast *S. pombe*.**

11.5 Chromosome segregation

Segregation of condensed chromosomes depends on centromeres.

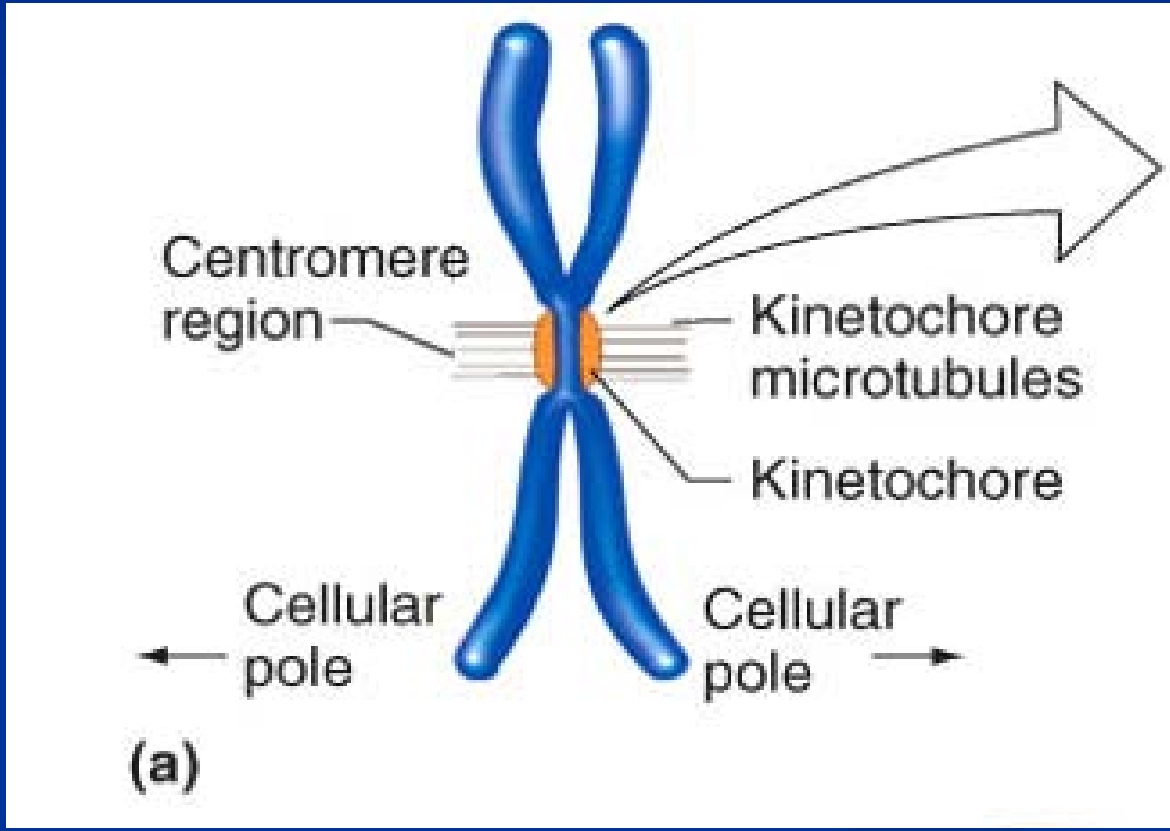


Fig. 11.22

- **Centromeres appear as constrictions on chromosomes.**
  - **Contain blocks of repetitive, simple noncoding sequences called satellite DNAs.**
  - **Satellite DNAs consist of short sequences 5 - 300 bases in length and have different higher-order packaging than other regions.**
  - **Histone H3 is replaced by a histone variant called CENP-A in eukaryotes.**

Yeast centromeres

Yeast centromere: ~120 bp
Human centromere: ~1,000,000 bp

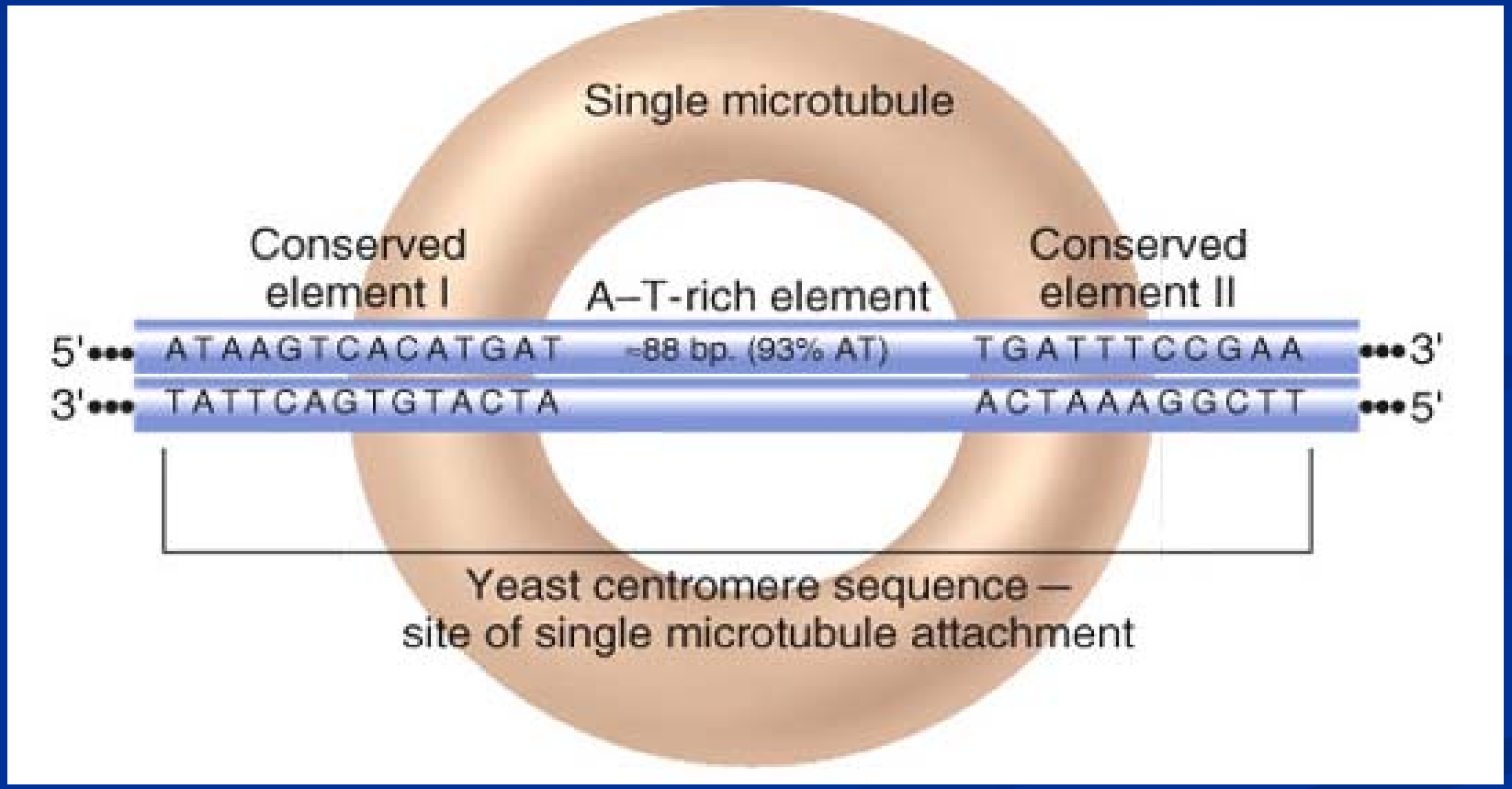
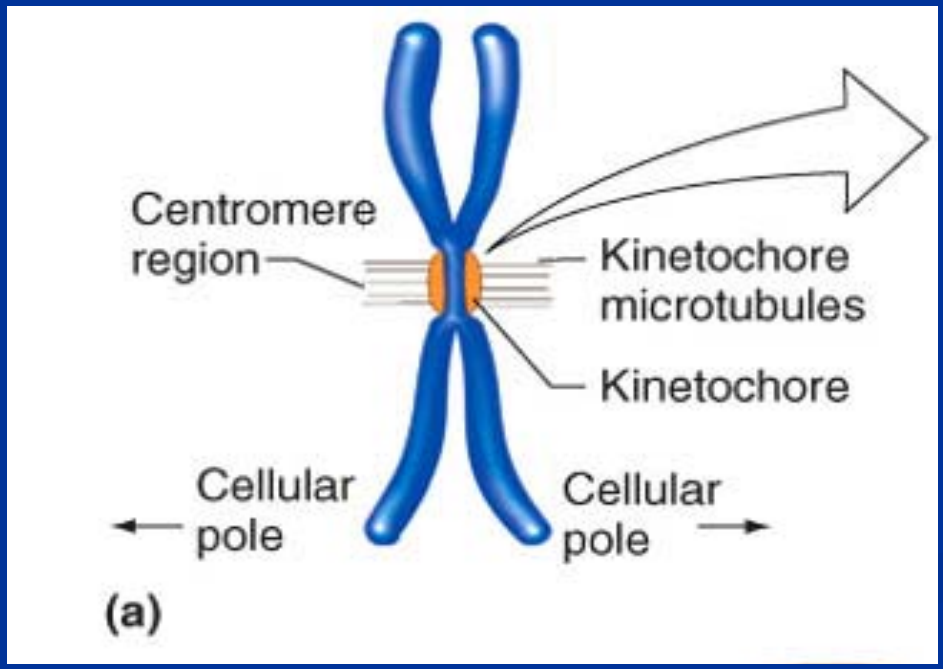


Fig. 11.23

Centromeres have two functions:

- Hold sister chromatids together.
- Facilitate chromosome segregation.
  - **Kinetochore** – a specialized structure composed of DNA and protein that is the site at which chromosomes attach to the spindle microtubules.



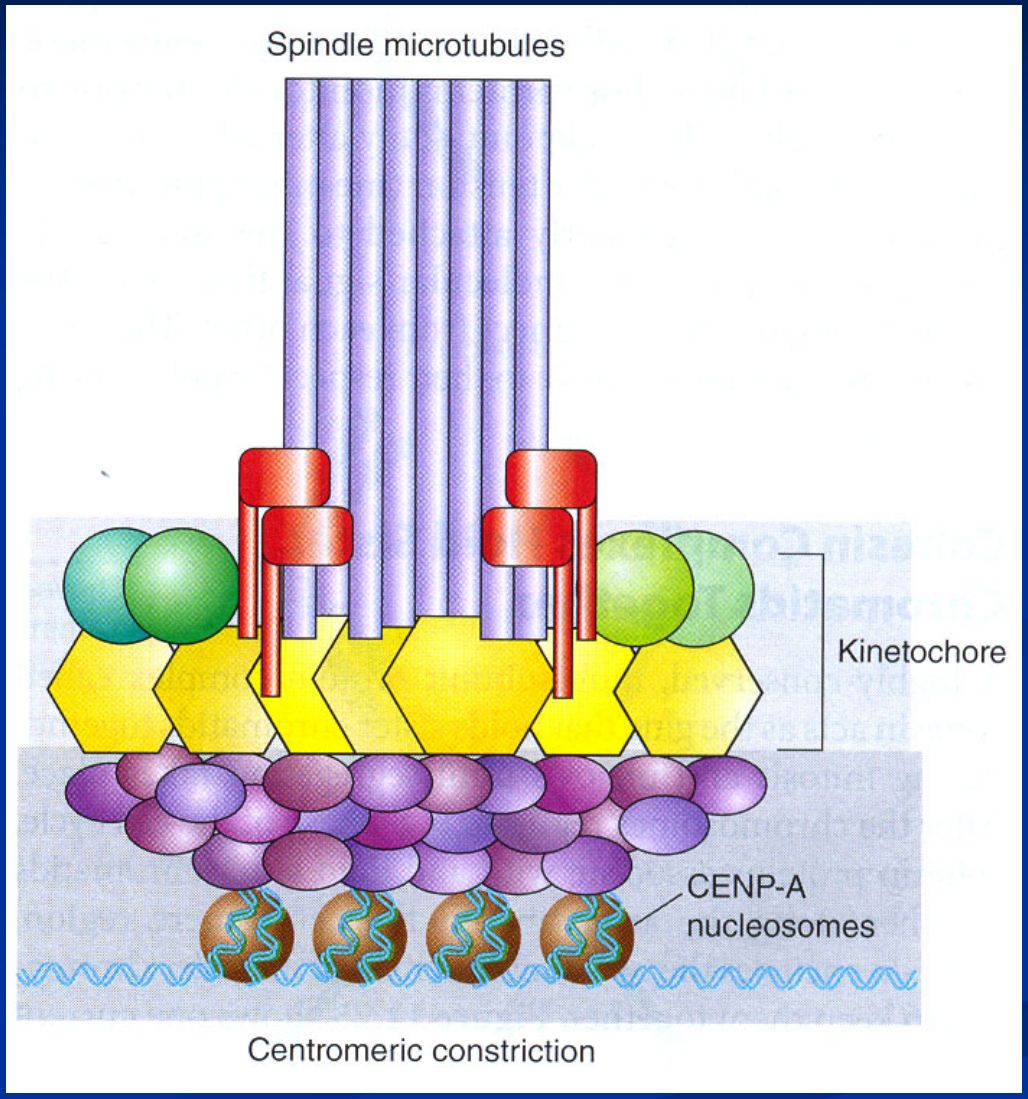
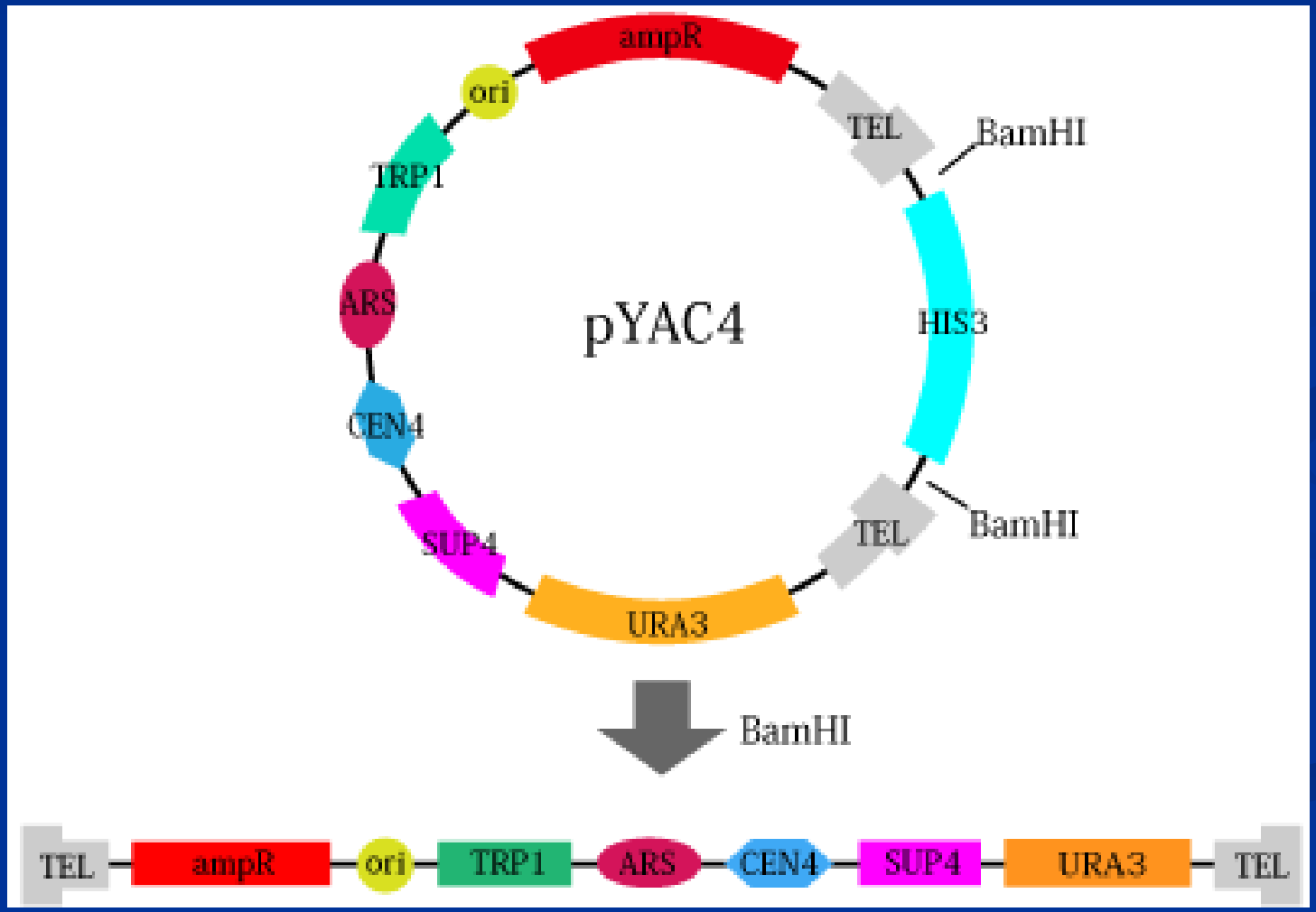
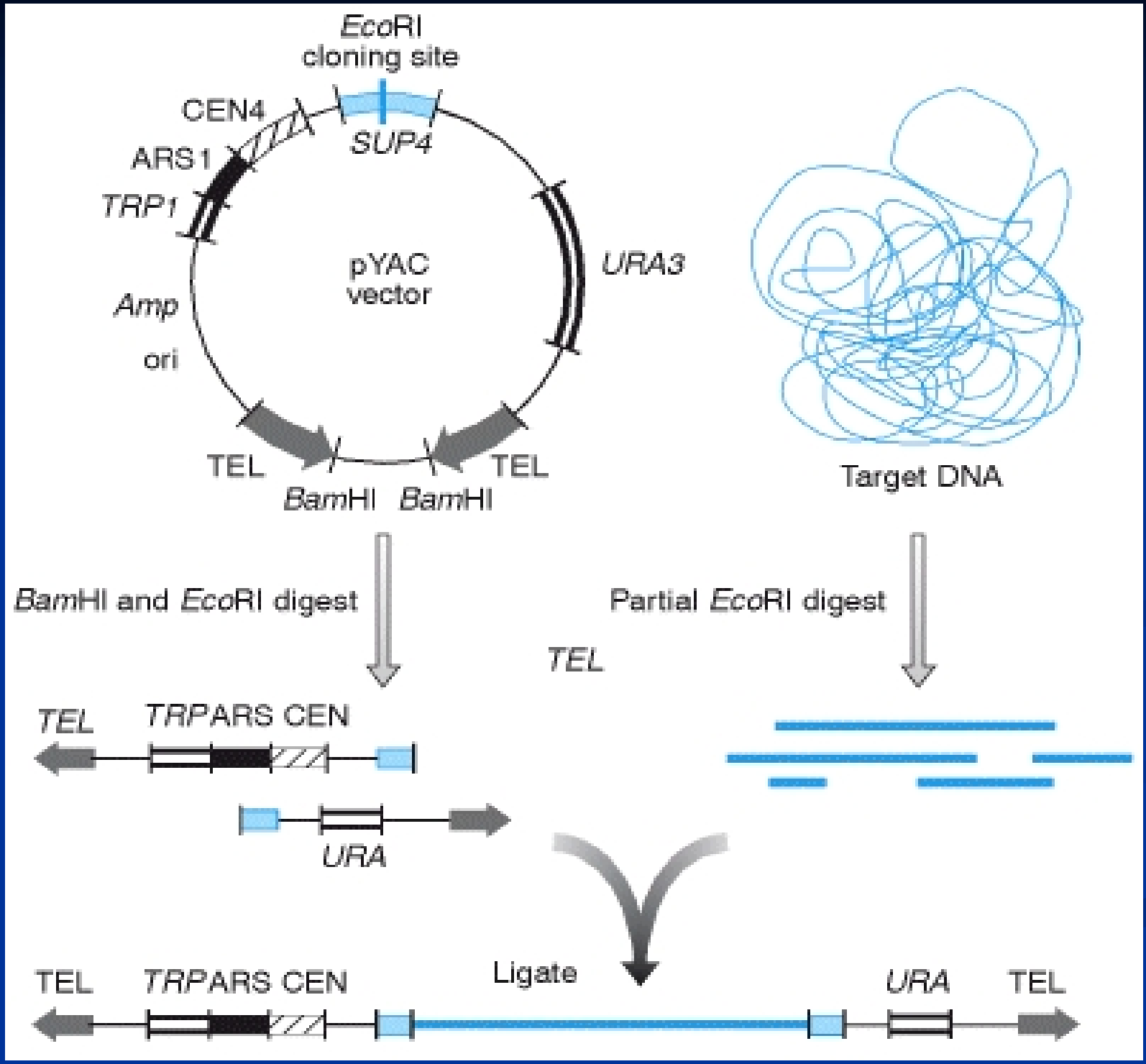


Fig. 11.22, 11.24

11.6 Artificial chromosomes

- YACs (yeast artificial chromosomes), constructed in 1980s.
  - Insert size 250-2,000 kb




EcoRI
cloning site
CEN4
ARS1
TRP1
SUP4
URA3
pYAC
vector
Amp
ori
TEL
TEL
BamHI
BamHI
Target DNA
BamHI and EcoRI digest
Partial EcoRI digest
TEL
TEL
TRPARS CEN
URA
Ligate
TEL
TRPARS CEN
URA
TEL