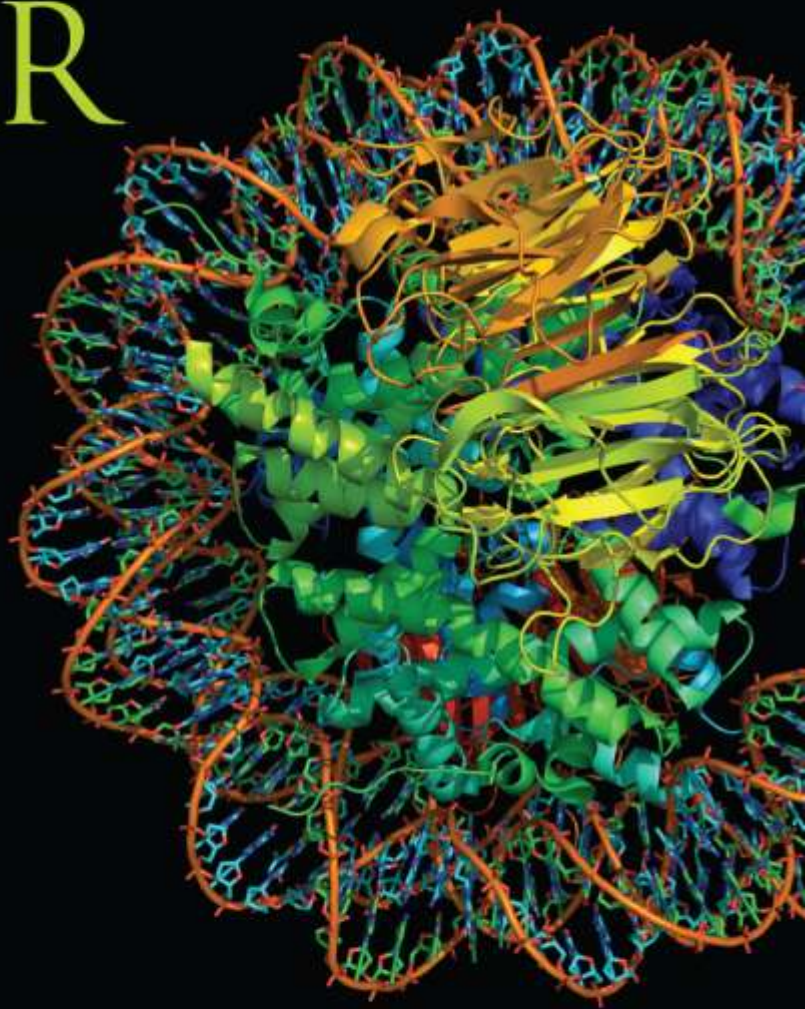
Principles of

MOLECULAR BIOLOGY

BURTON E. TROPP



Chapter 5
Chromosomes

CHROMOSOMES

DR. NABIL BASHIR

Chromosomes

In order to compact DNA molecules to fit inside a cell, specific proteins interact with DNA

- Forms a condensed nucleoprotein complex called **Chromatin**

Bacterial Chromatin

- *E. coli* DNA is a closed circle
  - Length ≈ 1,600 μm
  - Fits into a cell 0.5 μm in diameter x 1μm long
  - DNA forms a condensed nucleoprotein complex called the **nucleoid**

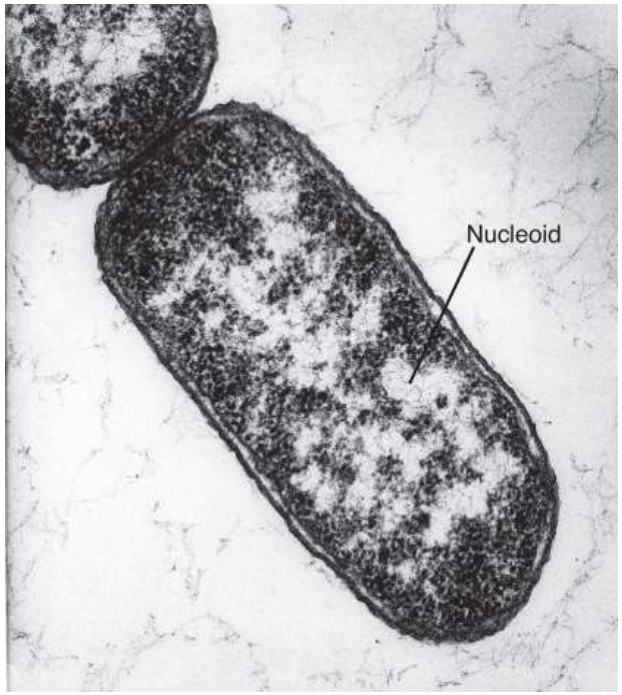


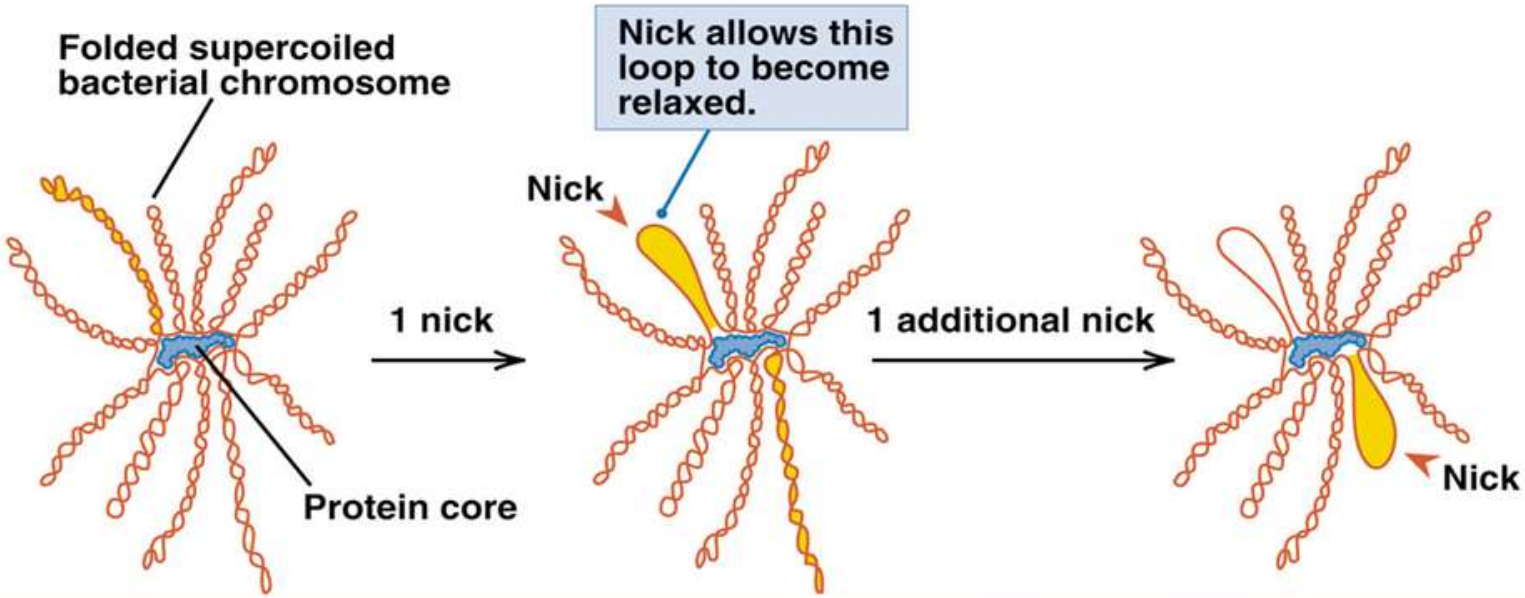
Figure 05.01: An electron micrograph of a thin-section of Escherichia coli. The nucleoid is the light region.

(Photo courtesy of the Molecular and Cell Biology Instructional Laboratory Program, University of California, Berkeley.)

Bacterial Chromatin

- Electron micrograph of released *E. coli* DNA reveals chromatin loops
  - Mostly supercoiled DNA loops with some relaxed loops (caused by nicks)
  - Each loop appears to be insulated from the others
  - Current model proposes supercoiled loops attached to a protein core
    - *E. coli* estimated to have 400 loops
    - Each loop is topologically independent

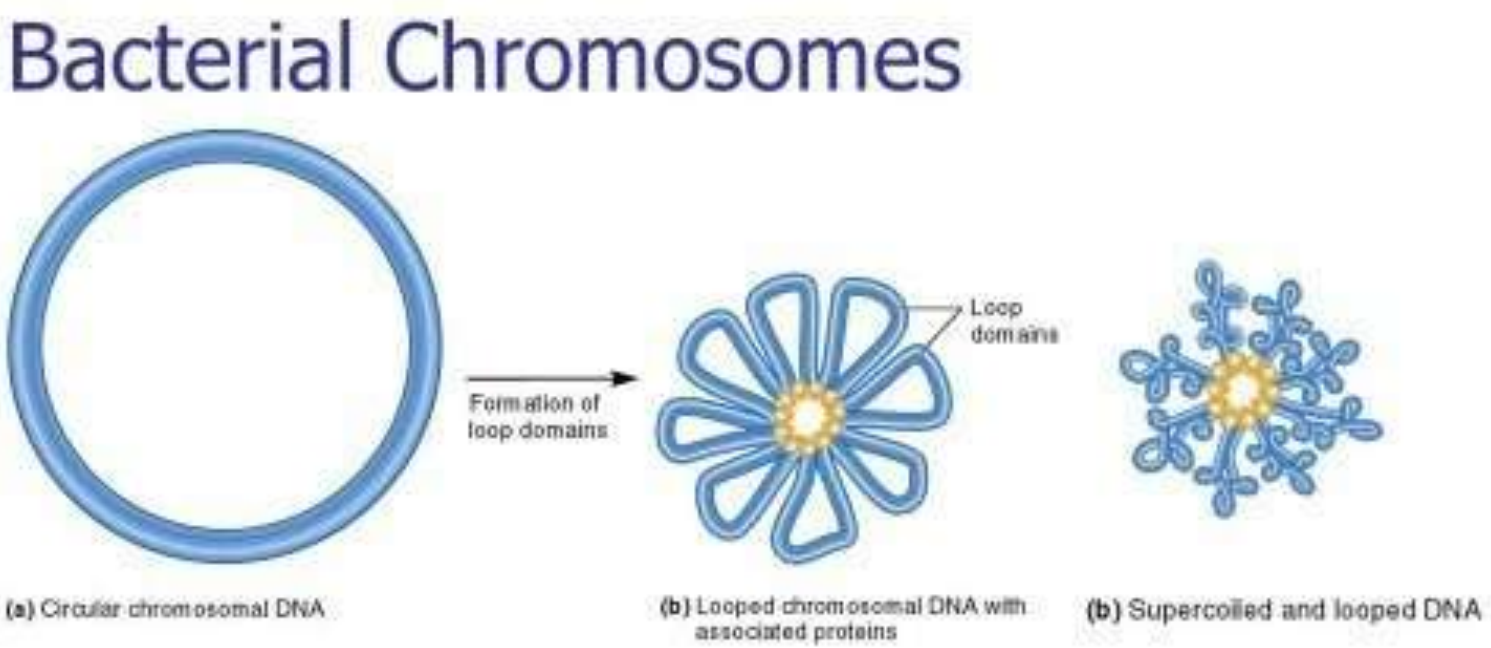
Mechanism of folding of a bacterial chromosome



There are many supercoiled loops (~100 in *E. coli*) attached to a central core. Each loop can be independently relaxed or condensed.

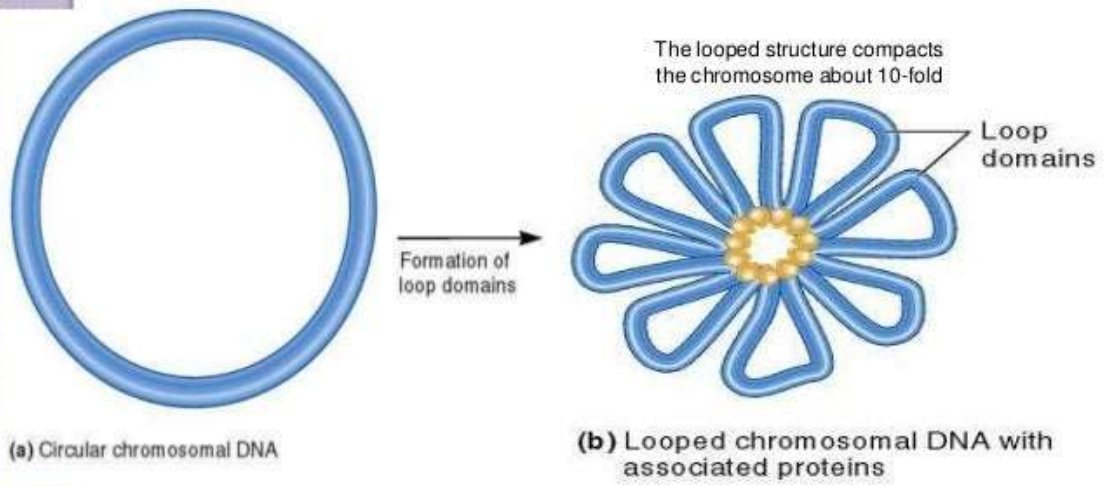
Topoisomerase enzyme – (Type I and II) that introduce or remove supercoiling.

Mechanism of folding of bacterial chromosome



- Chromosomal DNA is compacted ~ 1000 fold to fit within cell

To fit within the bacterial cell, the chromosomal DNA must be compacted about a 1000-fold This involves the formation of loop domains. The number of loops varies according to the size of the bacterial chromosome and the species.
E. coli has 50-100 with 40,000 to 80,000 bp of DNA in each.



- DNA supercoiling is a second important way to compact the bacterial chromosome

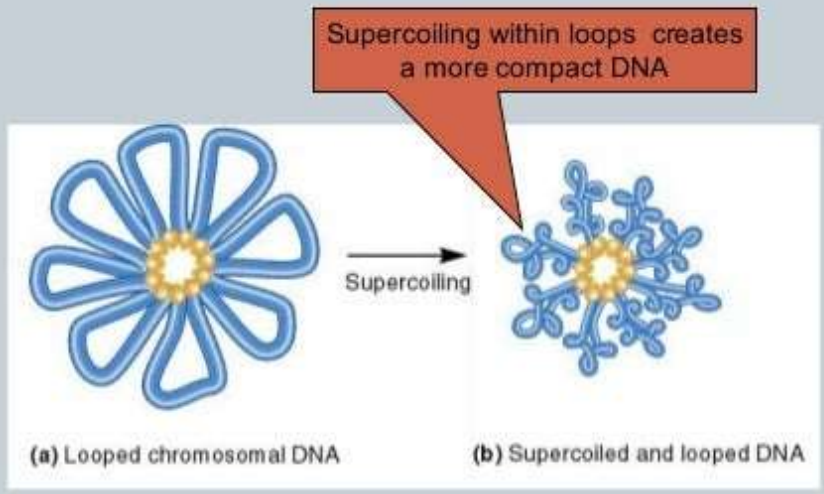


Figure 10.6

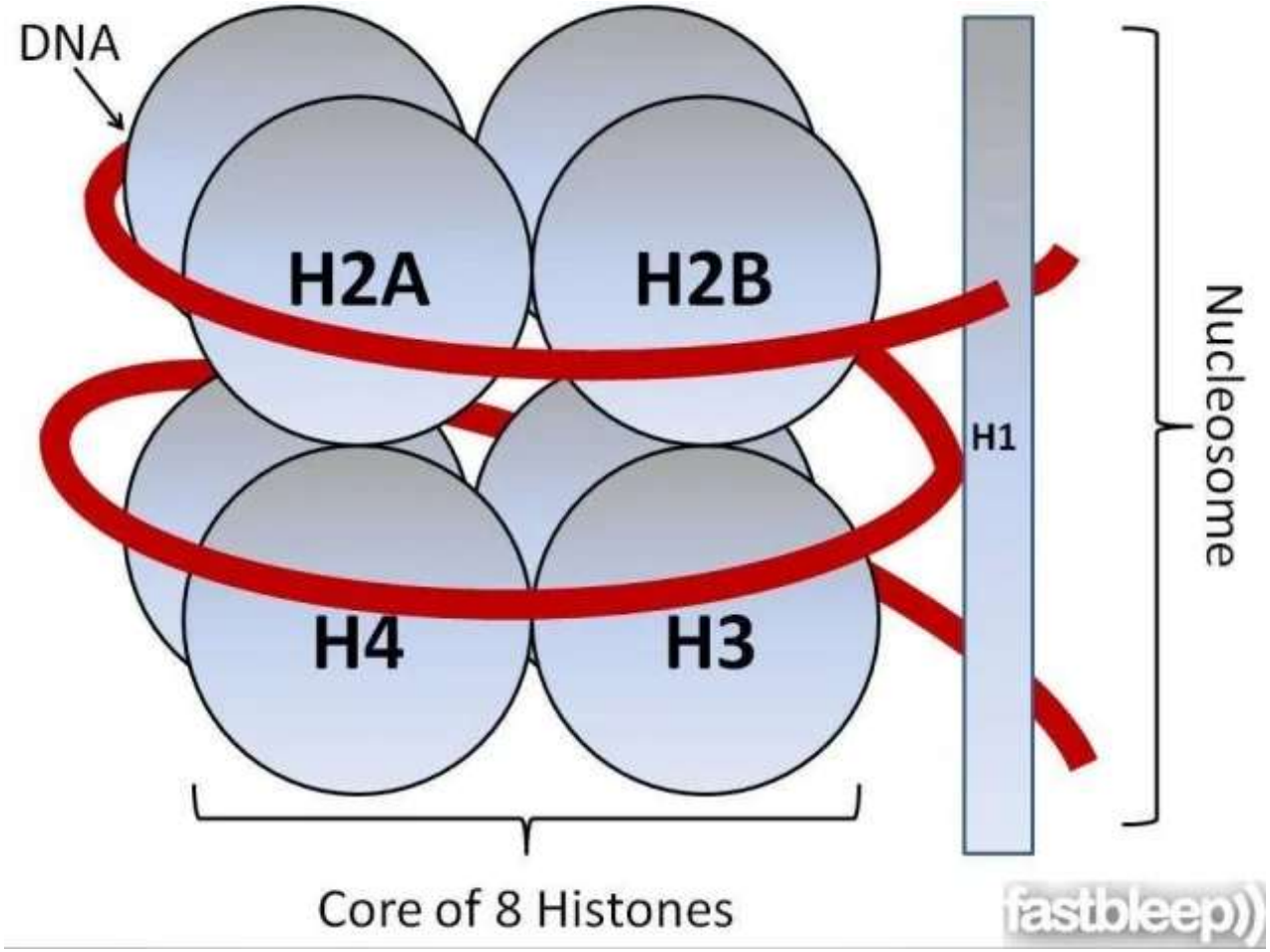
- Figure 10.7 provides a schematic illustration of DNA supercoiling

Eukaryotic Chromatin

- Highly condensed structures called **chromosomes**
  - Germ cells (reproductive cells) have a characteristic number of chromosomes (n) also called the haploid number
  - Somatic cells are diploid (2n)

The Nucleosome

- Multiple levels of chromatin packing
  - First level of organization: interactions between DNA and histones
  - Chromatin contains 5 major classes of histones
    - Each histone has a high percentage of the basic amino acids lysine and arginine



The Nucleosome

TABLE 5.1 Histone Composition

Histone	Molecular Mass (kDa)	% Lysine	% Arginine	% Lysine + Arginine
H1	~21.0	29	1.5	30.5
H2A	14.5	11	9.5	20.5
H2B	13.7	16	6.5	22.5
H3	15.3	10	13.5	23.5
H4	11.3	11	14.0	25.0

The Nucleosome

- Histones are electrostatically attracted to the negatively charged phosphates of DNA
  - Attraction can be broken by high salt concentration
  - Purified DNA and purified histones mixed together will reconstitute chromatin
  - Extremely well conserved proteins

The Nucleosome

- Uncondensed chromatin from interphase nuclei resemble beads on a string
  - Bead is a nucleoprotein complex called a **nucleosome**
    - DNA wound around 8 histone protein core
    - Treatment with micrococcal nuclease cleaves DNA in the linker region to release free nucleosomes

The Nucleosome

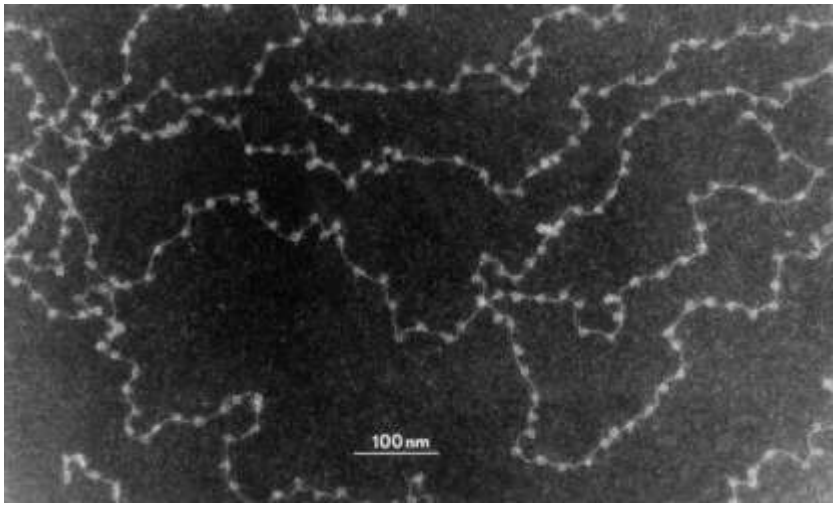


Figure 05.12: Electron micrograph of chromatin. The beadlike nucleosome particles have diameters of approximately 11 nm.

(© Ada & Donald Olins/Biological Photo Service.)

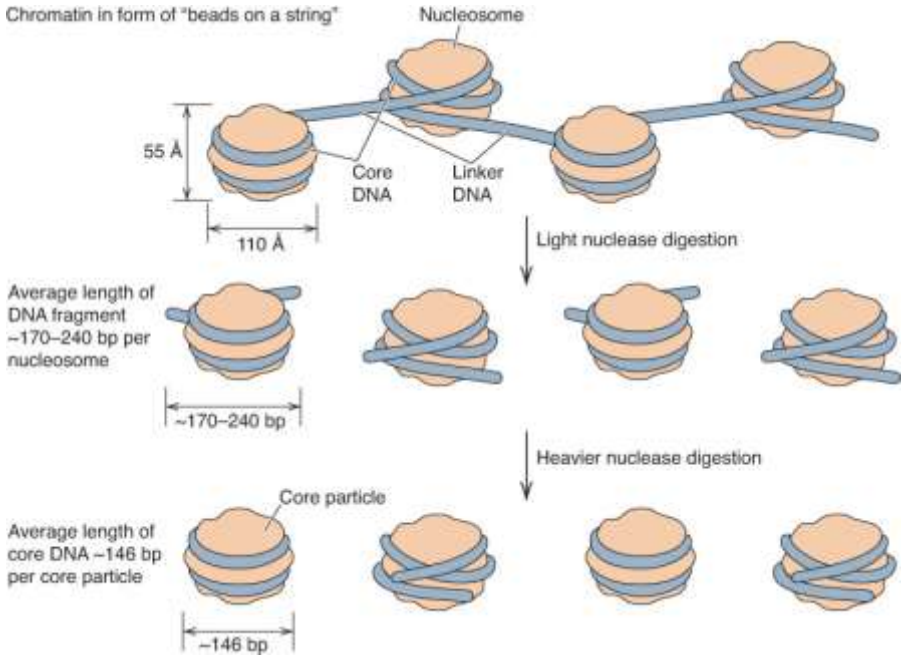


Figure 05.13: "Beads on a string" structure of chromatin.

The Nucleosome

- Free nucleosomes can be further digested to the nucleosome core particle made up of
  - 164bp DNA
  - Octameric protein complex
    - 2 copies each of: H2A, H2B, H3 and H4

The Nucleosome

- X-ray crystallography reveals the atomic structure of nucleosome core particles
  - Histone octamer has two-fold symmetry
  - DNA is negatively supercoiled in the nucleosome
  - A-T rich minor grooves tend to contact the histone octamer

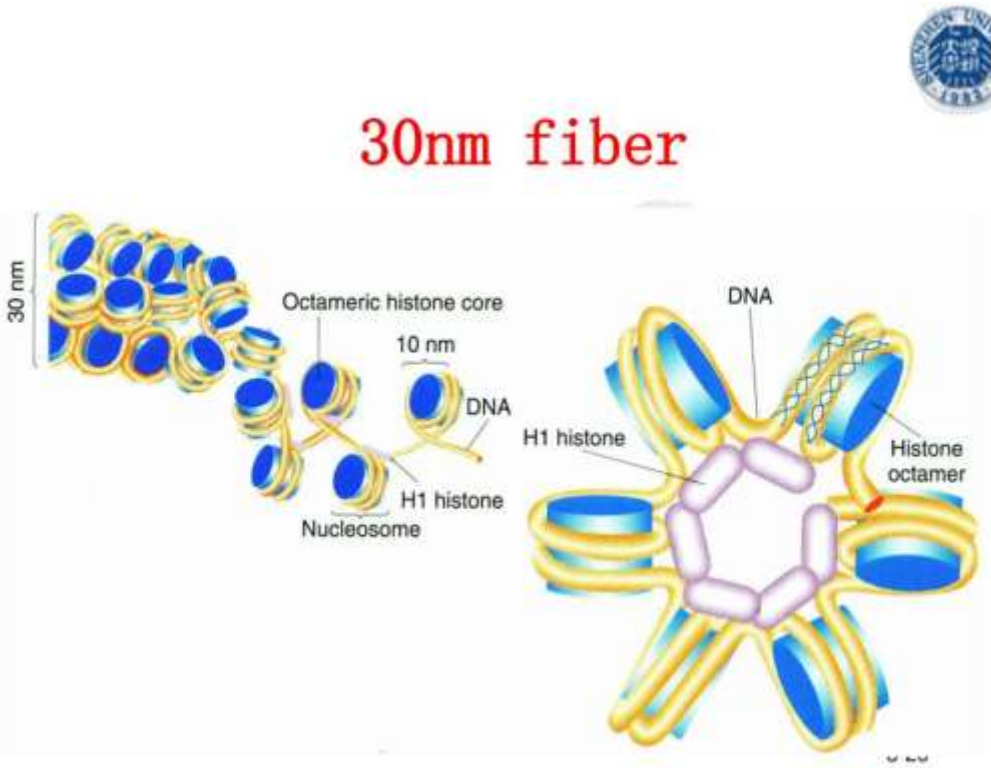
The Nucleosome

- Nature of H1 interaction and the core particle is not precisely known
  - When the fifth histone (H1) is present the particle is called the **chromatosome**
    - 166bp DNA wrapped around octameric histone core held in place by H1

The next level of chromatin organization is not yet resolved

30nm fiber

May be an artifact of conventional electron microscopy



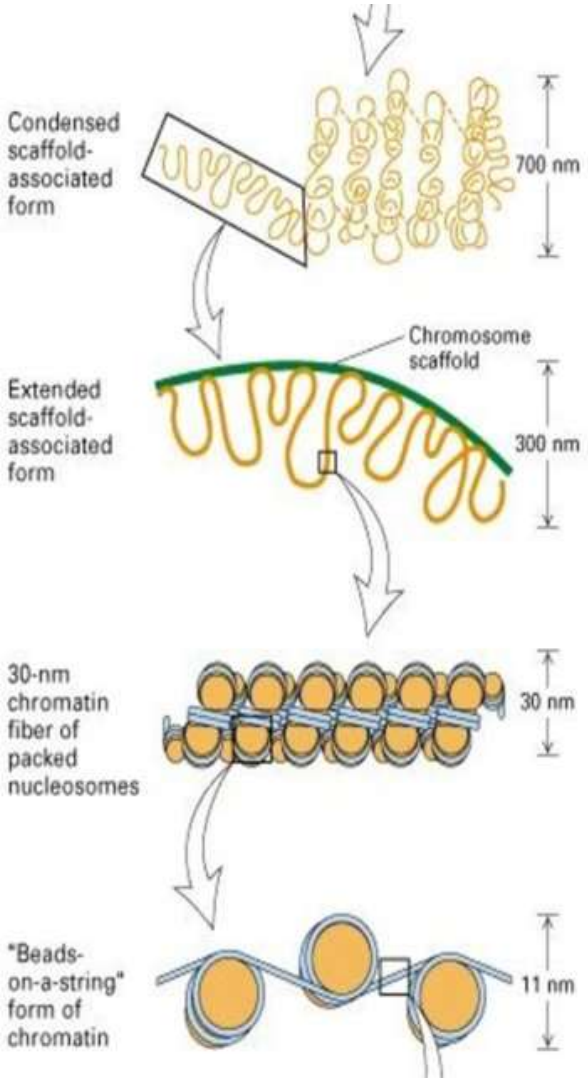
The Nucleosome

- Scaffold model
  - Predicts that non-histone proteins form a central scaffold along the long axis
    - Histone depleted chromosomes have DNA loops attached to a protein scaffold

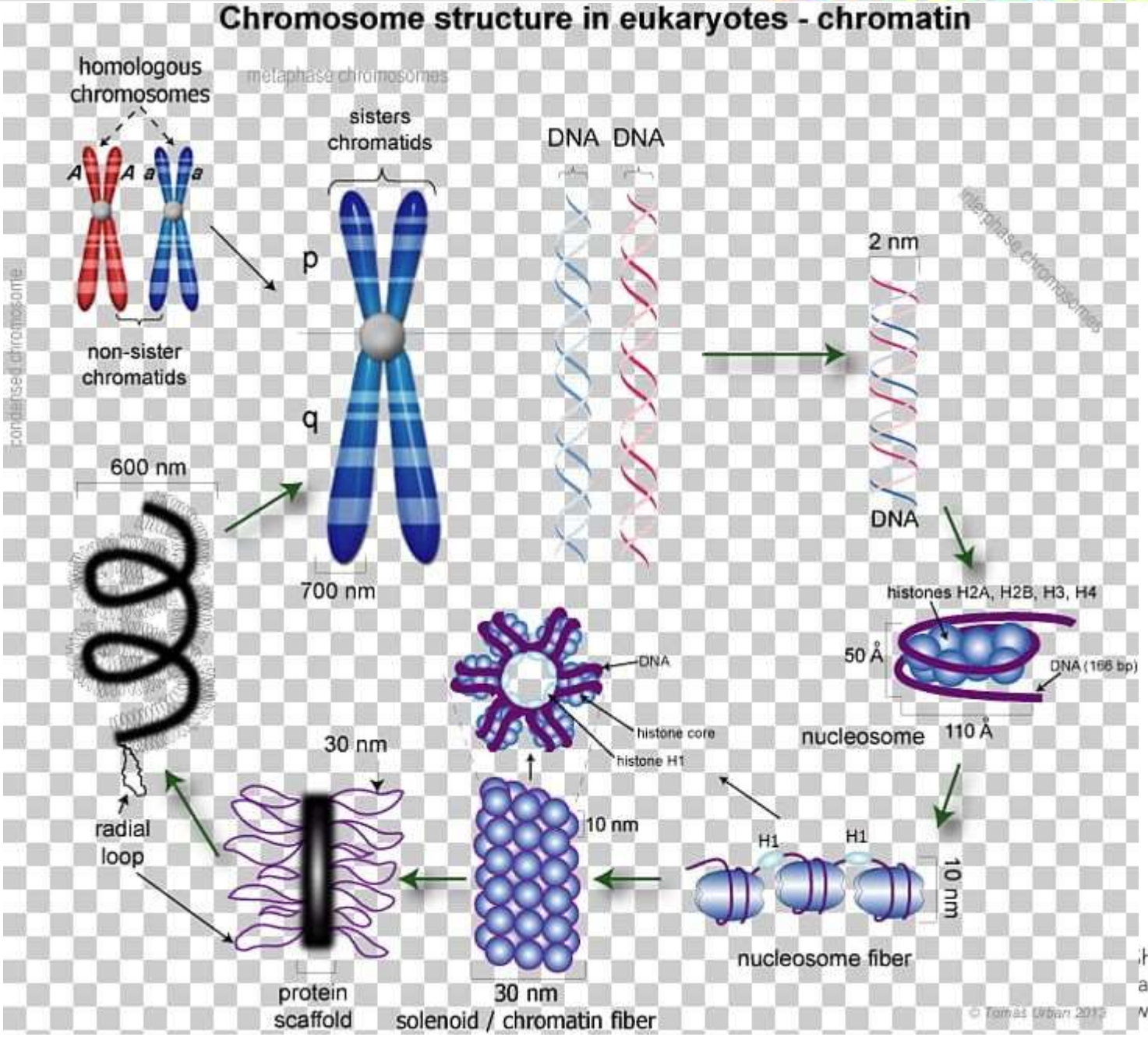
Scaffold model

Predicts that non-histone proteins form a central scaffold along the long axis

Histone depleted chromosomes have DNA loops attached to a protein scaffold



Chromosome structure in eukaryotes - chromatin



The Telomere

- Chromosome end has 3’ overhang
- Single strand ranging from 100’s bp in yeast to 1,000’s bp in vertebrates
  - Folds back to form a loop

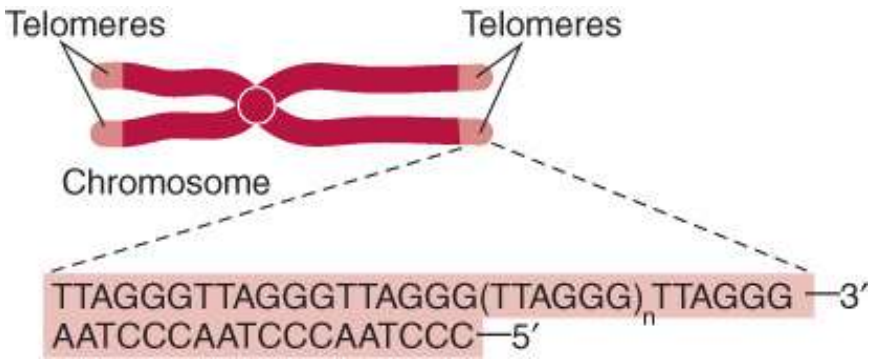
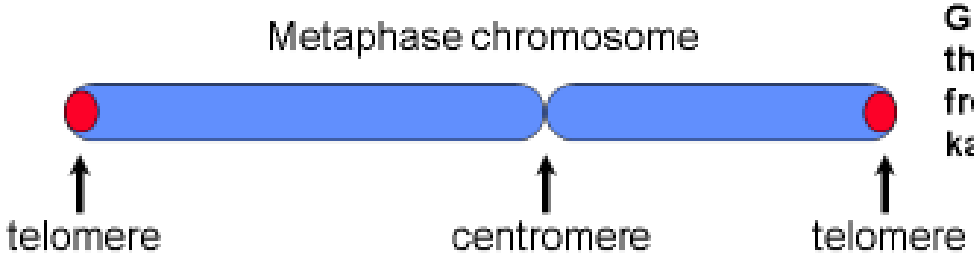


Figure 05.23: 3′-Telomere overhang. Telomeres, shown in pink, are located at the ends of the sister chromatids. Each telomere has a G-rich 3′-overhang.

Telomeres and aging

Telomeres are protective "caps" on chromosome ends consisting of short 5-8 bp tandemly repeated GC-rich DNA sequences, that prevent chromosomes from fusing and causing karyotypic rearrangements.



- telomerase (an enzyme) is required to maintain telomere length in germline cells
- most differentiated somatic cells have decreased levels of telomerase and therefore their chromosomes shorten with each cell division

The Telomere

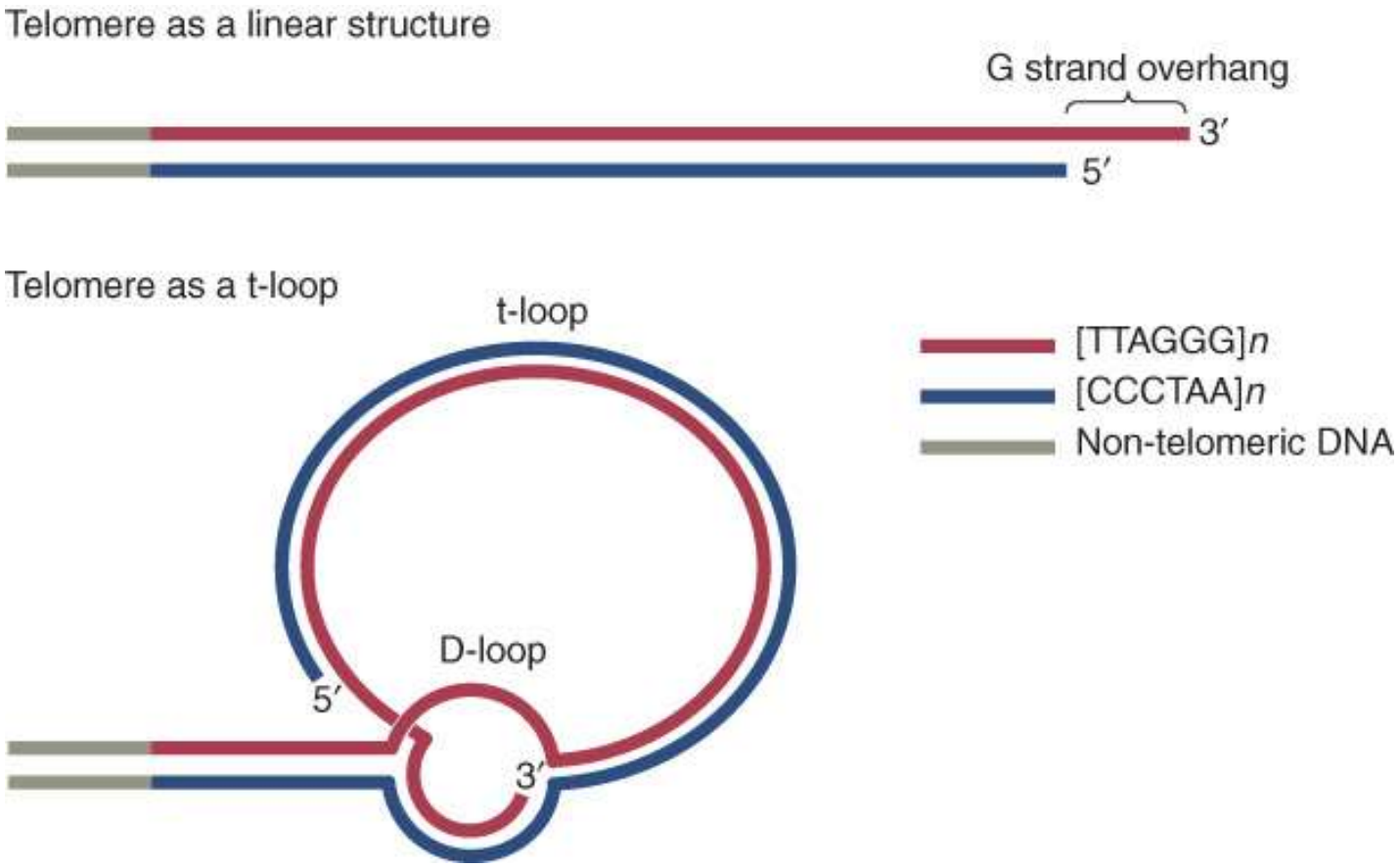
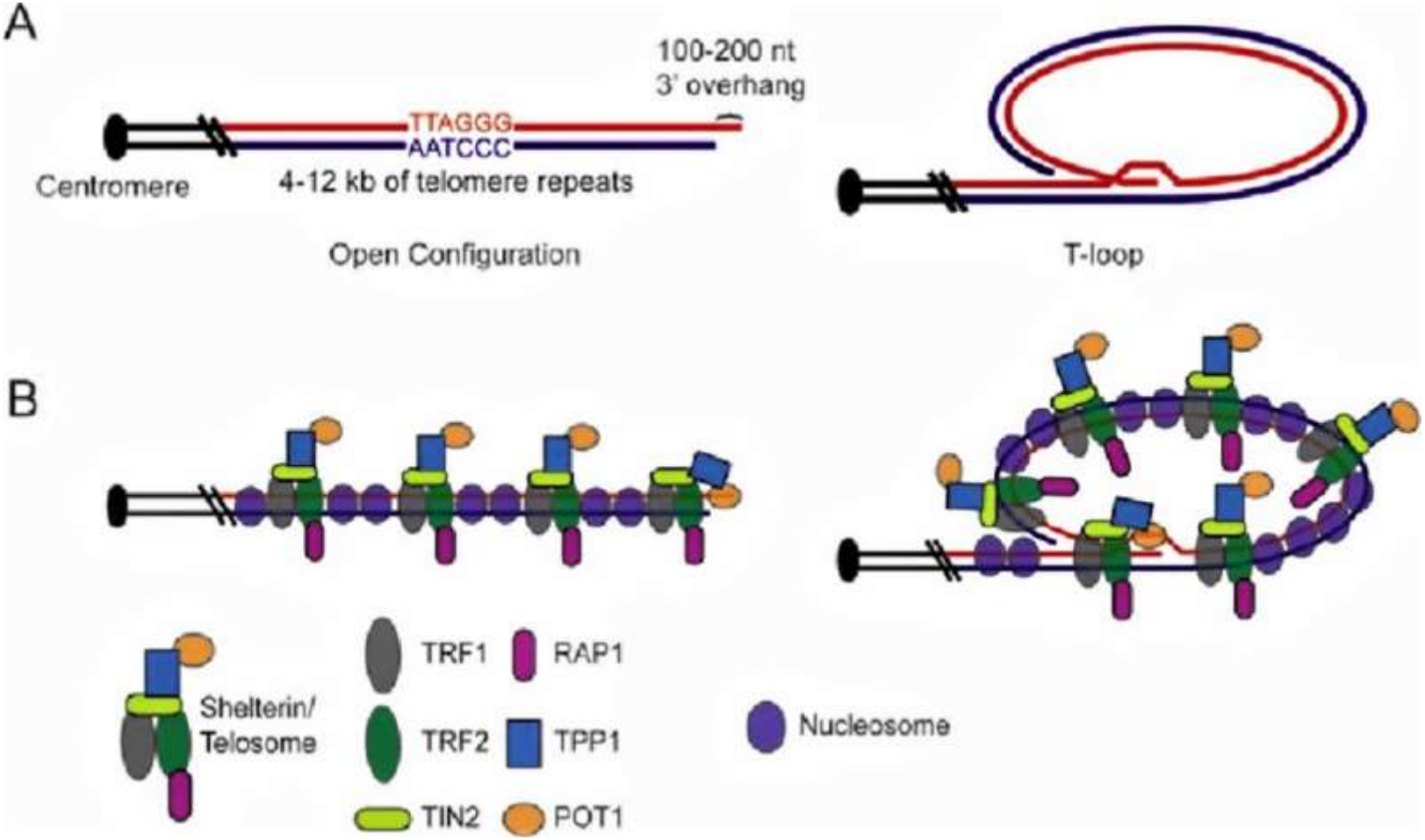


Figure 05.24: Structure of telomere t- and D-loops.

(Adapted from Lundblad, V. 2000. Science 288:2141–2142.)



The Telomere

- Werner syndrome helicase
  - Protein involved in telomerase formation
  - Defective in Werner syndrome
    - A genetic disease associated with premature aging
- Eukaryotic chromosomes lose DNA from their ends during replication
    - Require the enzyme telomerase
    - Failure to replace telomeres is potentially responsible for finite number of divisions of cells in culture
    - Most cancer cells have telomerase