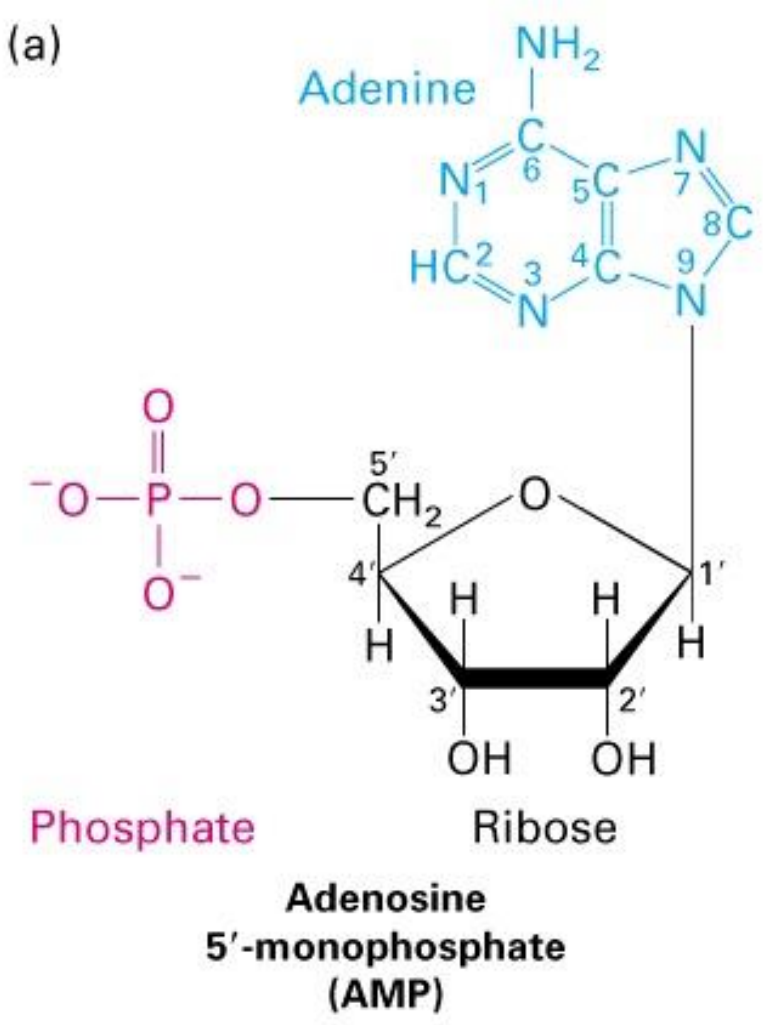
Genes and chromosomes

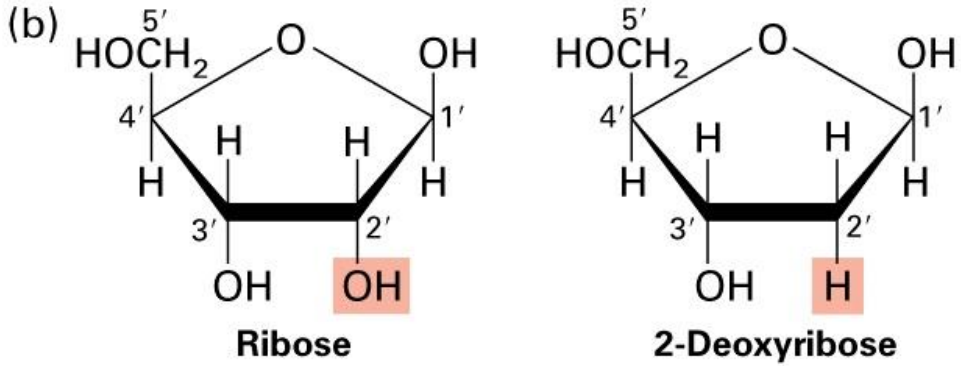
Dr.Rula Abdul-Ghani

All nucleotides have a common structure

A nucleotide present in RNA



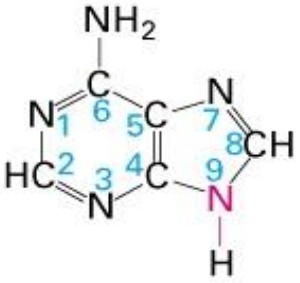
The pentoses in nucleic acids



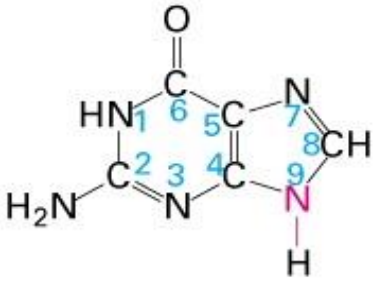
There are five principal bases in nucleic acids

Nitrogen 9 of purines and 1 of pyrimidines are bonded to C1 of ribose or deoxyribose

PURINES

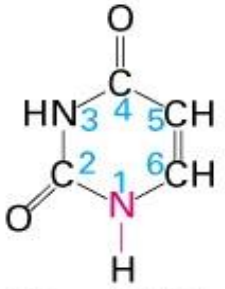


Adenine (A)

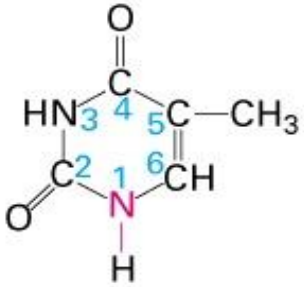


Guanine (G)

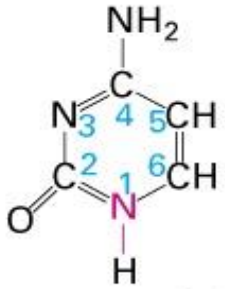
PYRIMIDINES



Uracil (U)



Thymine (T)



Cytosine (C)

A, G, T, C are present in DNA

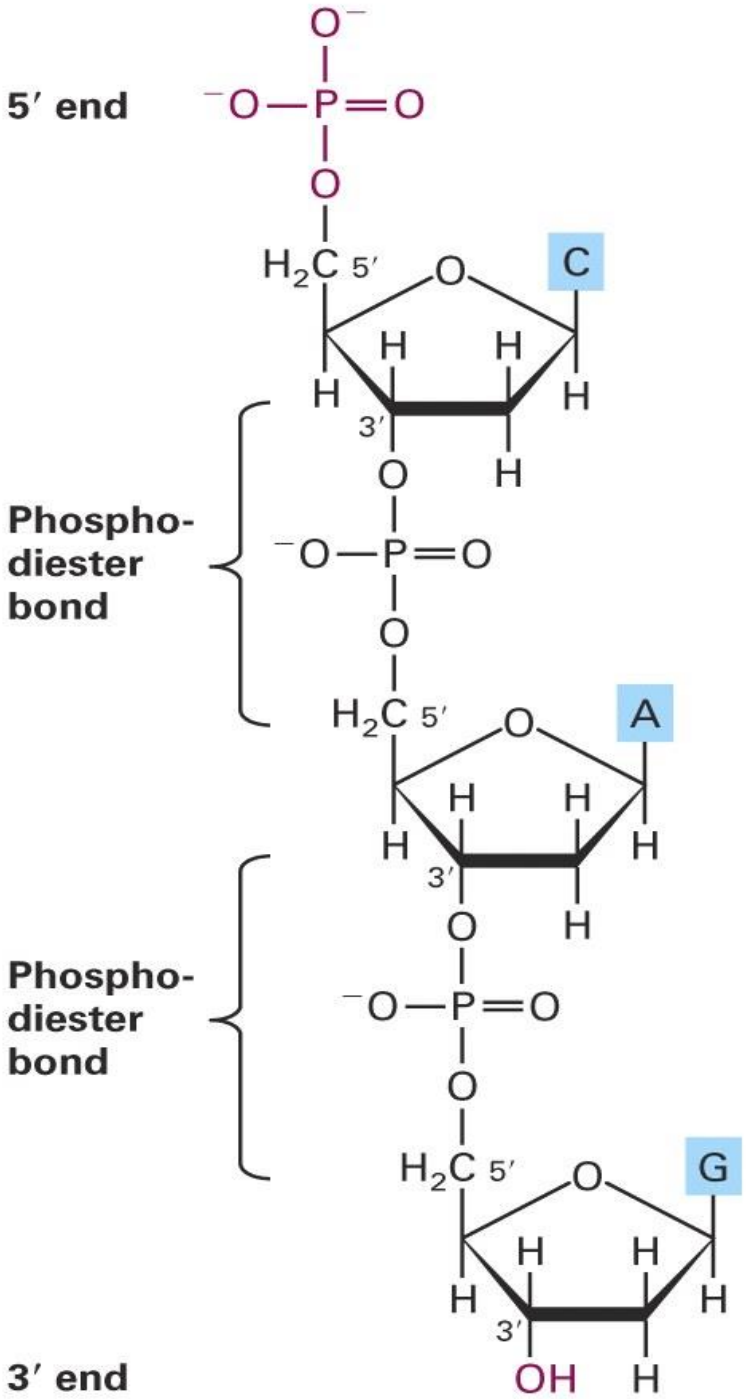
A, G, U, C are present in RNA

Nucleotide subunits are linked together by phosphodiester bonds

This nucleic acid chain is represented as (5’ C-A-G 3’)

always in 5’ to 3’ direction left to right.

5’ phosphate group on the 5’ of the sugar and a 3’ hydroxyl of the sugar

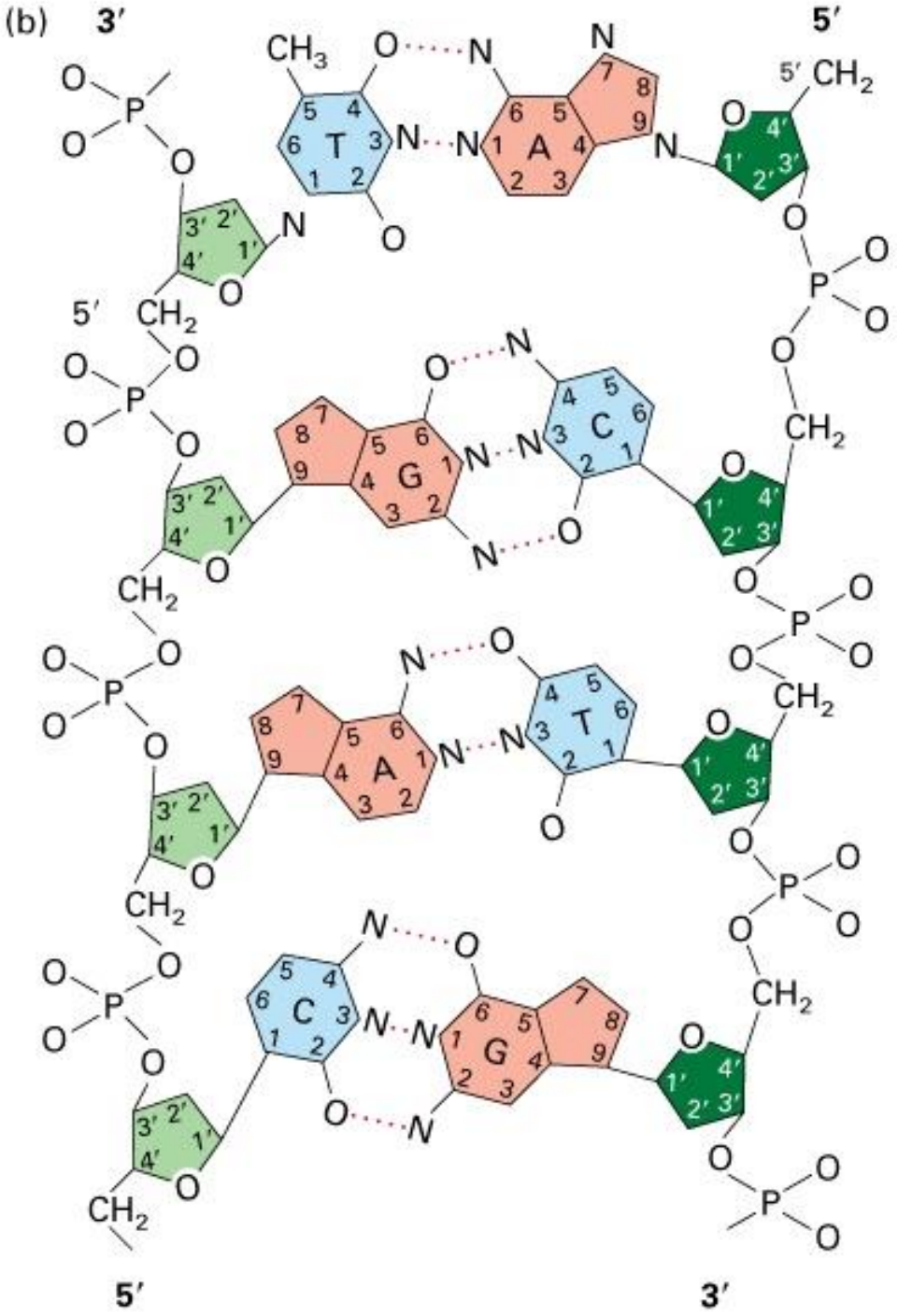


Native DNA is a double helix of complementary antiparallel chains held together by:

1- Hydrogen bonding between complementary base pairs (A-T or G-C)

2- Hydrophobic interactions between planar bases/ stacked adjacent bases.

This contributes to the stability of the double helix.



Forces that maintain DNA as a double strand….

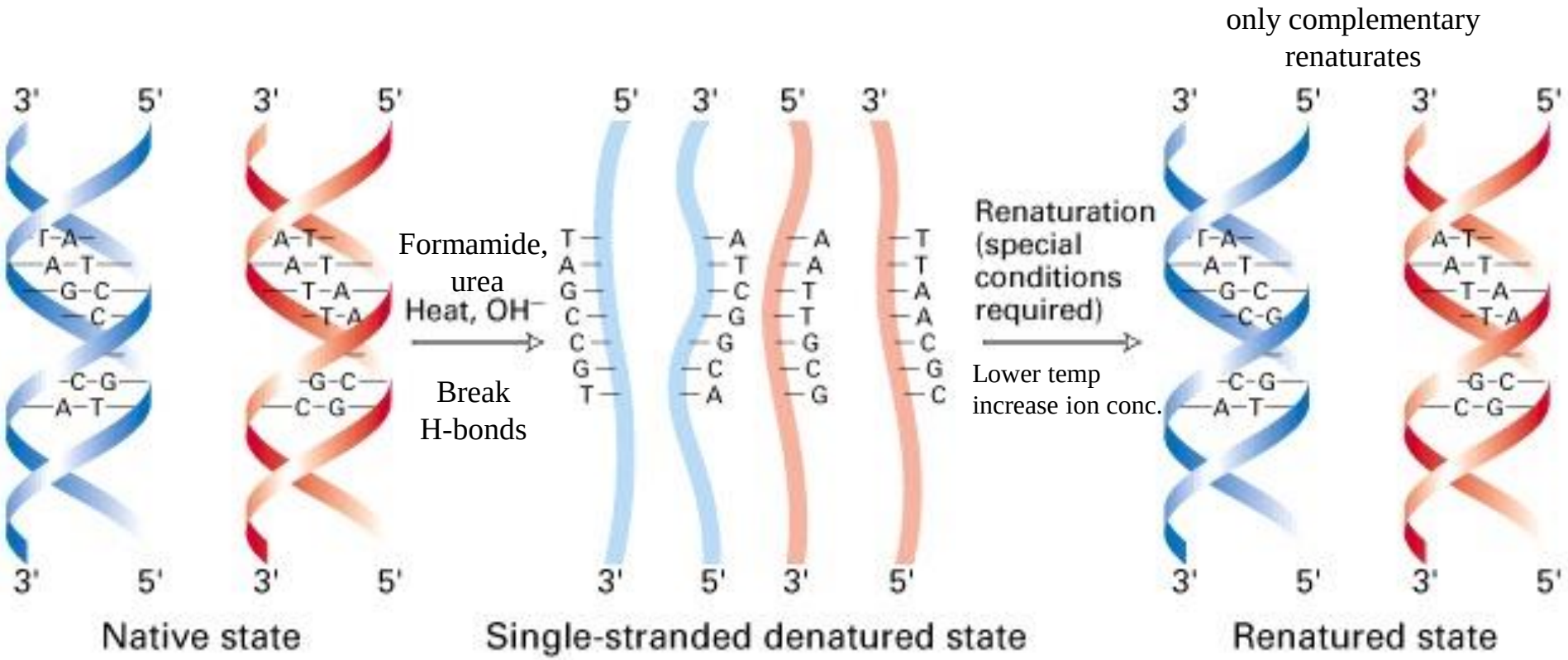
H-bonding

Hydrophobic interactions (cooperative base stacking)

DNA at room temp. 25◦C and pH 7 is viscous, formamide, ↑pH (NaOH), ↑ temperature → sharply destroy viscosity.

DNA can undergo reversible strand separation

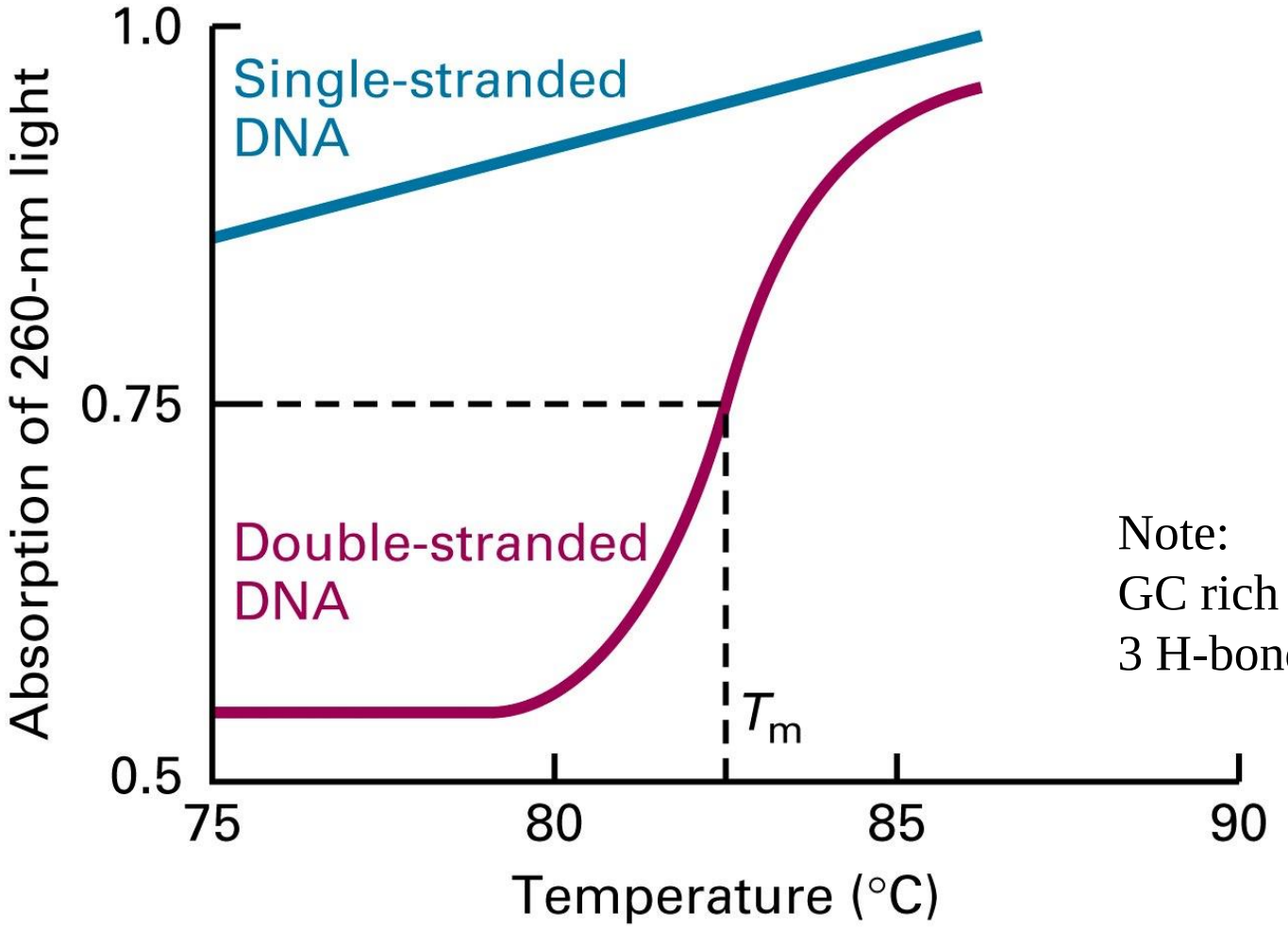
denaturation/ melting = Unwinding and separation of the double strand



DNA denaturation changes its absorption to UV at 260 nm (used to measure DNA concentration.

DNA denaturation extent dependent on time, DNA conc., ionic content of soln.

Analysis of DNA denaturation



Note:
GC rich higher Tm:
3 H-bonds to break.

T_m= temperature at which ½ the bases in a dsDNA sample have denatured

- DNA denaturation and renaturation is the

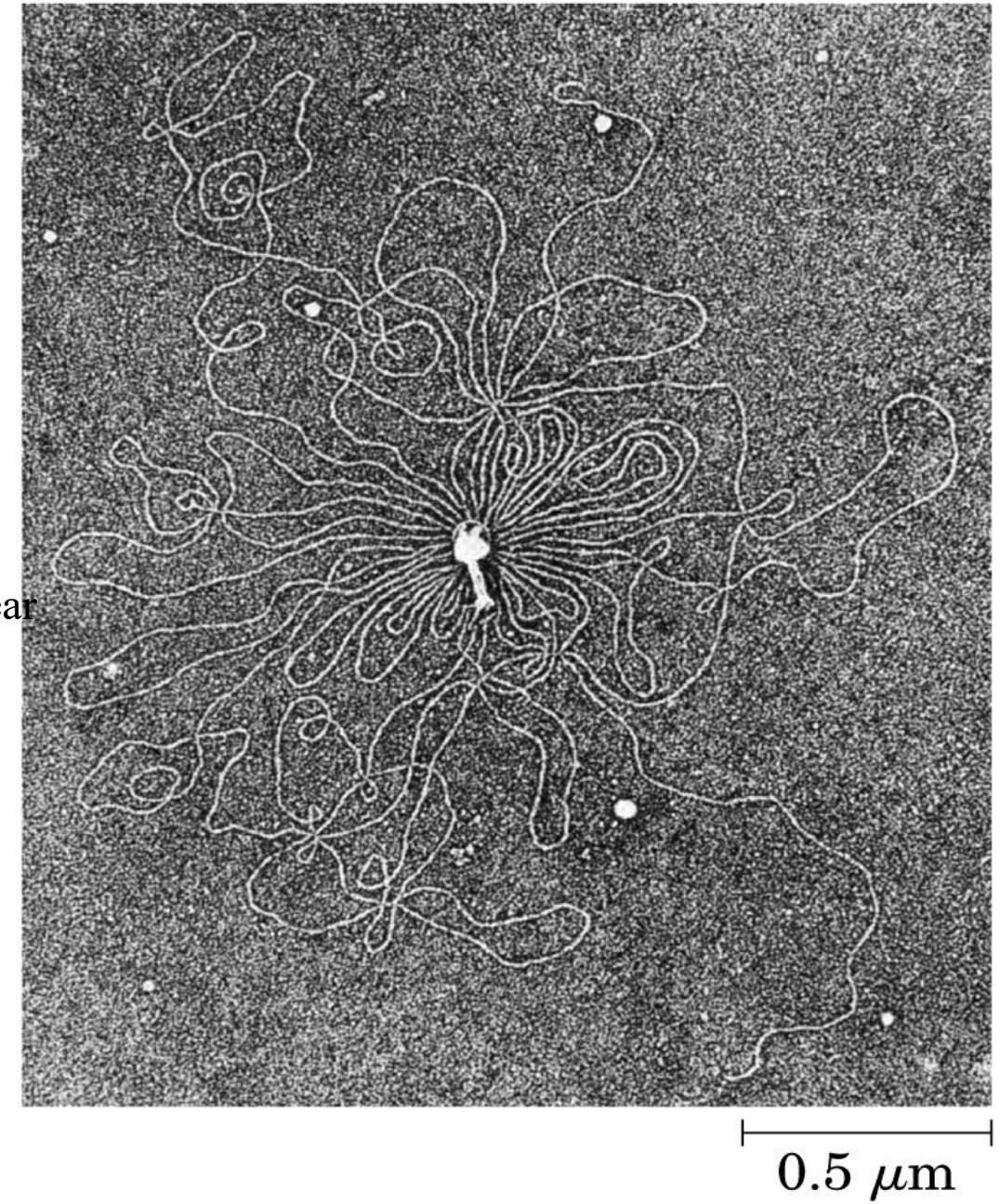
basis of nucleic acid hybridization and PCR = a powerful technique in Molecular studies.

DNA much longer than the cells or viral packeges that contain them.

Bacteriophage T2 lysed in distilled water allowing DNA to spread on water.

- protein coat surrounded by its single linear DNA .

•Normally in undamaged T2, DNA is packaged in the phage head.



Viruses :

Infectious parasites, use the resources of the host has a DNA / RNA genome surrounded by a protein coat.

Although viral genomes are small the length of their DNAs is much greater than the long dimensions of the viral particles that contain them.

table 24–1

The Sizes of DNA and Viral Particles for Some Bacterial Viruses (Bacteriophages)

Virus	Number of base pairs in viral DNA*	Length of viral DNA (nm)	Long dimension of viral particle (nm)
ϕX174	5,386†	1,939†	25
T7	39,936	14,377	78
λ (lambda)	48,502	17,460	190
T4	168,889	60,800 (290 x longer)	210

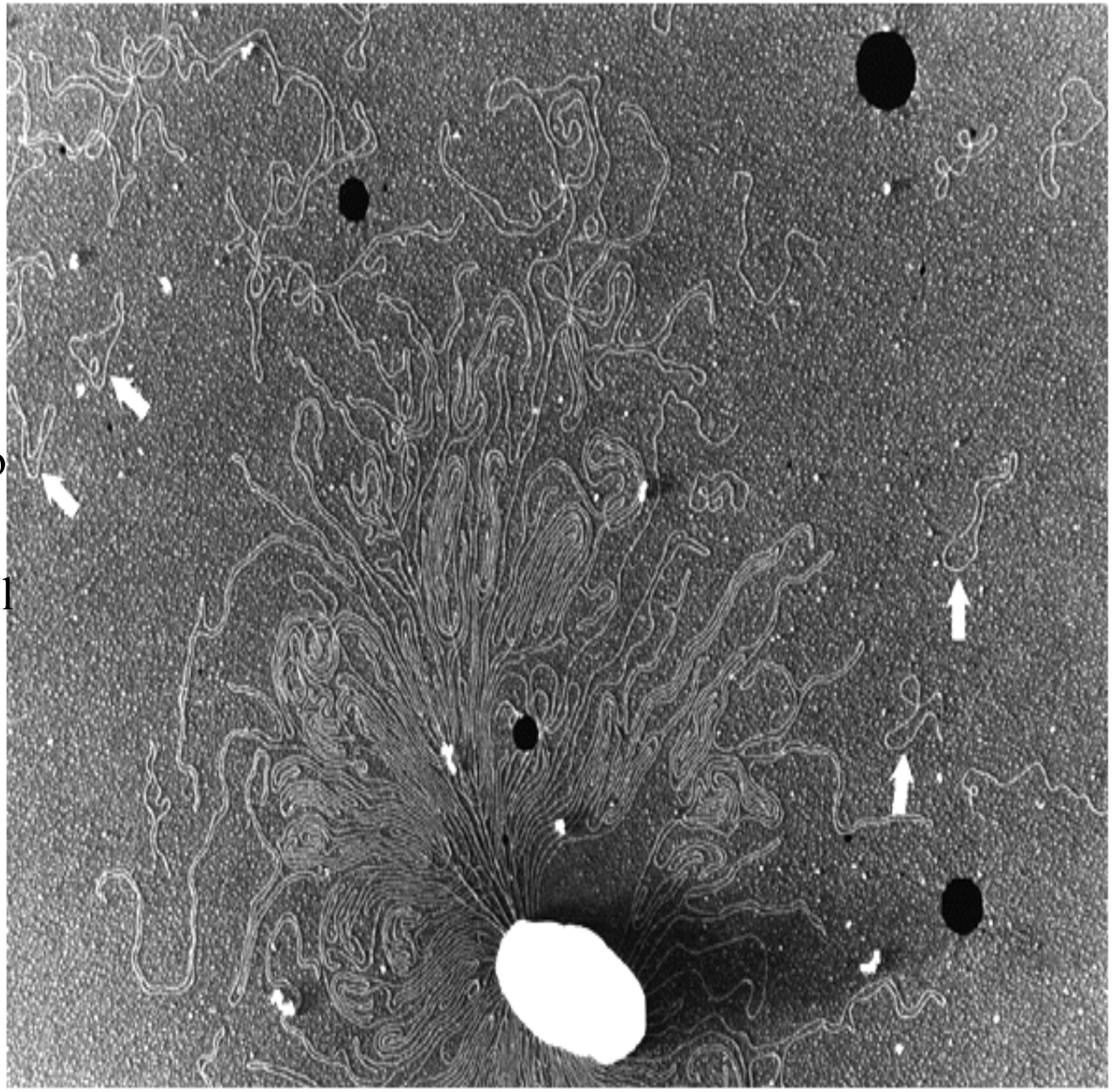
* The complete base sequences of these bacteriophage genomes have been determined.

†Data are for the replicative form (double-stranded).

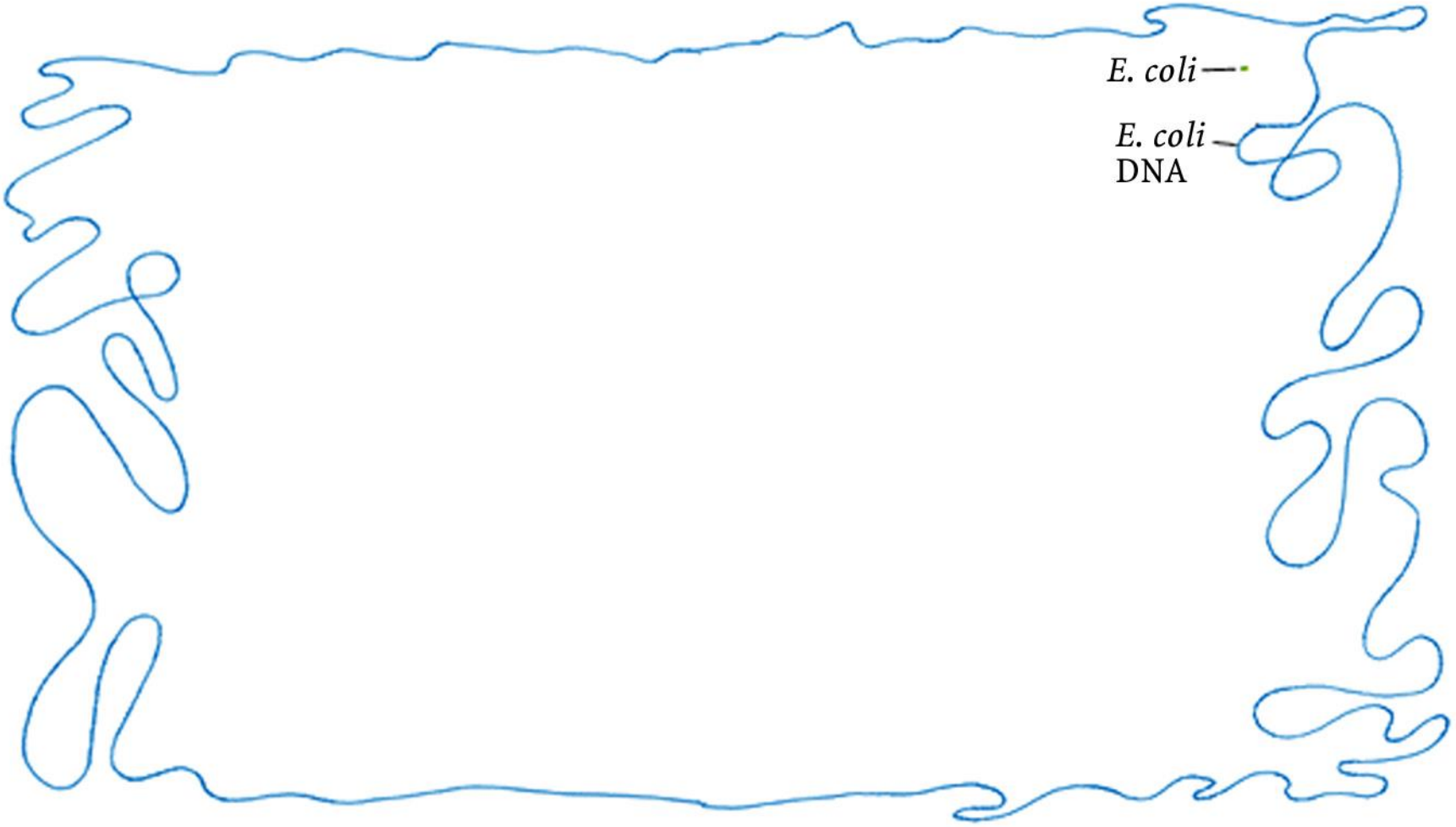
Bacteria:

- E. coli 100> DNA bacteriophage lambda.
-One large circular DNA in <u>nucleoid + plasmids</u>
extrachromosomal DNA
a few thousands bp long
function self propagation into daughter plasmids
pass into daughter cells at cell division
-Some carry genes important for bacterium
e.g. antibiotic resistant.

DNA from a lysed E coli→



The length of the E coli chromosome(1.7mm) relative to the length of cell (2um).



Eukaryotes :

Yeast cell (simplest eukaryote)

2.6 times > DNA than E.coli

Fruit fly 35 times > E coli

Humans 700 times> E.coli

Genetic material in eukaryotes in chromosomes

table 24–2

Normal Chromosome Number in Some Organisms*

Bacteria	1	Honeybee (female)	32
Fruit fly	8	Fox	34
Red clover	14	Cat	38
Garden pea	14	Mouse	40
Yeast	16†	Rat	42
Maize (corn)	20	Rabbit	44
Frog	26	Human	46
Hydra	30	Chicken	78

*The diploid chromosome number is given for all eukaryotes except yeast.

†This is the haploid chromosome number for the yeast *Saccharomyces cerevisiae*. Wild yeast strains generally have eight (octoploid) or more sets of these chromosomes.

Eukaryotic chromosomes:

The DNA of human genome:

46 chromosomes in every somatic cell.

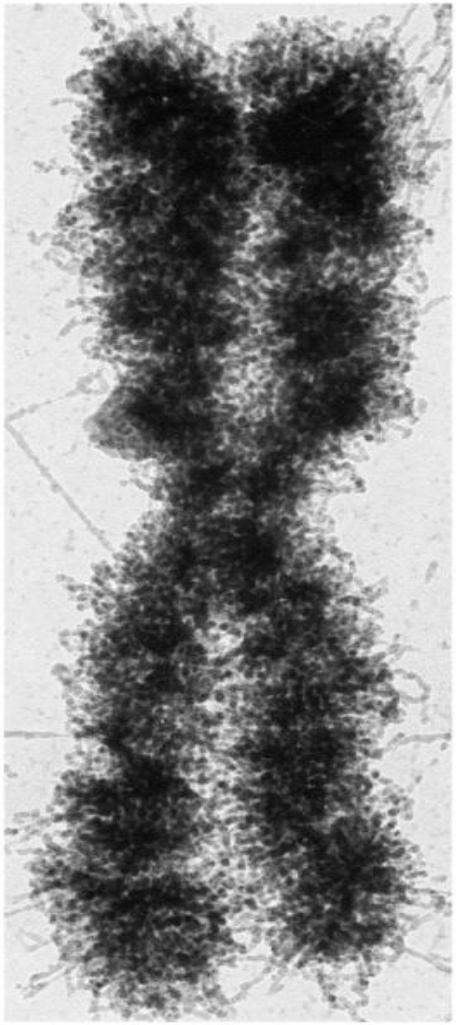
If placed end to end 2m DNA.

Adult human body= 10_{14} cell

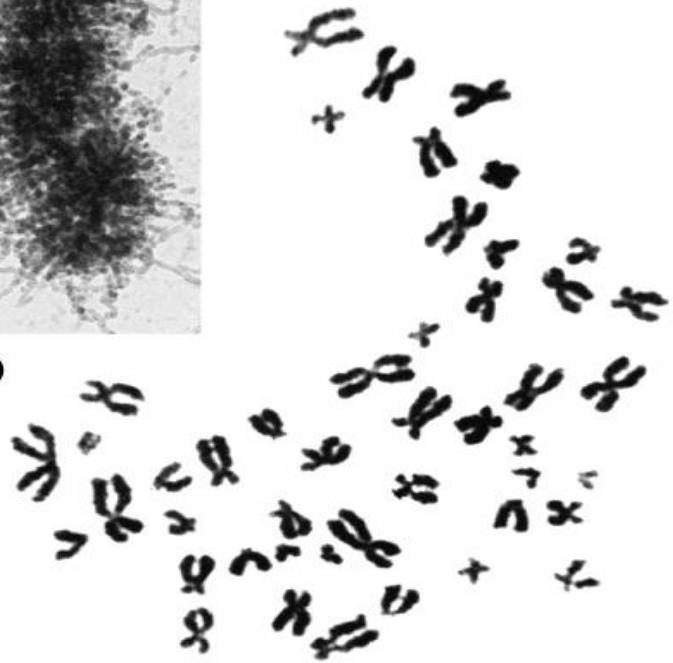
Length of DNA = 2 x 10_{11} km

Distance between Earth and sun

= 1.5x10_{8} km



(a)



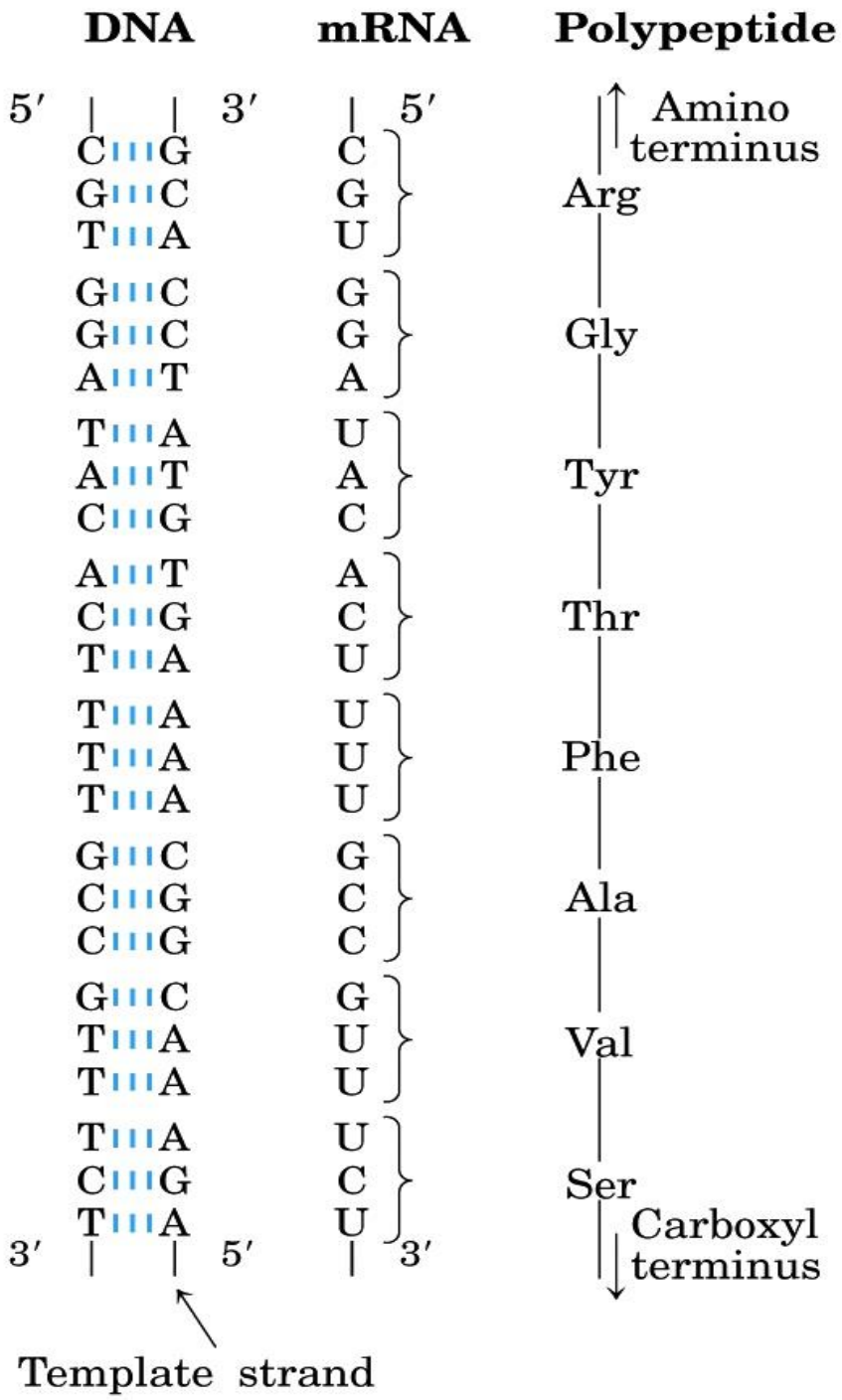
(b)

Genetic Code:

Triplets of nucleotide units in DNA determine the a.a in a protein through the intermediary mRNA.

One of the DNA strands serve as **template** for mRNA synthesis.

For polypeptide chain =
300 a.a corresponds to 900 bp



Gene =

A portion of chromosome that determines a single specific character / phenotype/ visible property.

A segment of genetic material that determines /codes for a protein / enzyme:

One gene one enzyme-------One gene one polypeptide.

Modern biochemical definition:

All the DNA that encodes the primary sequence of some final gene product (polypeptide / RNA).

How many genes in a single chromosome?

E coli (prokaryotic) = 4,6 million bp

encodes 4300 gene

Human = 3.2 billion bp

encodes 30,000- 35,000 genes

Introns:

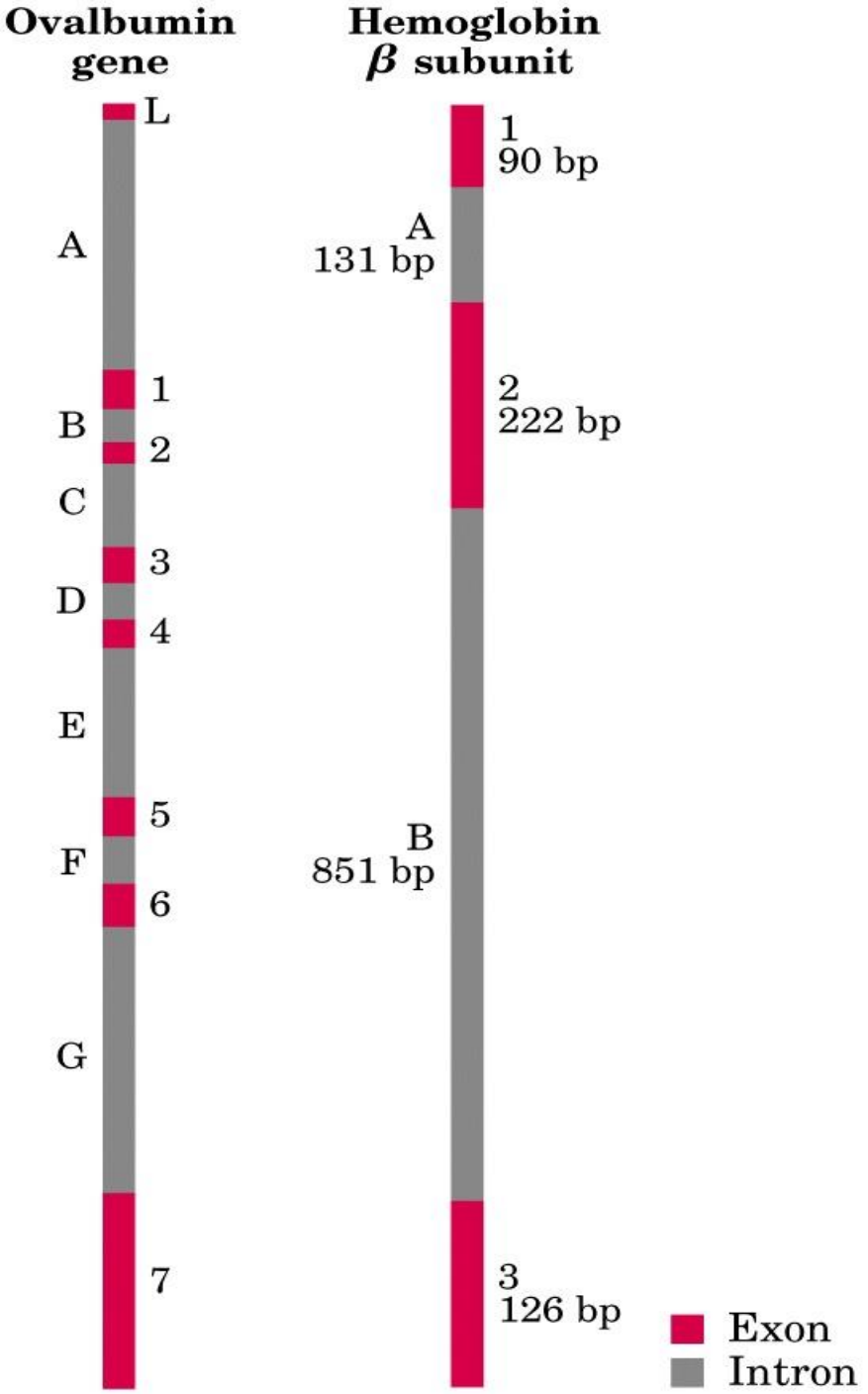
intervening sequences = nontranslated DNA segments in genes.

Exons:

a region of DNA within a gene transcribed to final (mRNA) molecule, rather than spliced out from transcribed RNA.

Introns in 2 eukaryotic genes:

Ovalbumin introns (7) much longer than exons (7+L) , introns=85% of the gene.



- **Satellite DNA** = Simple sequence DNA ~ 10bp

  highly repetitive DNA called because repetitions of short DNA sequence tend to produce a different frequency of nucleotides A, C, G and T and thus have a different density from bulk DNA - such that they form a second / 'satellite' band when genomic DNA separated on a density gradient.

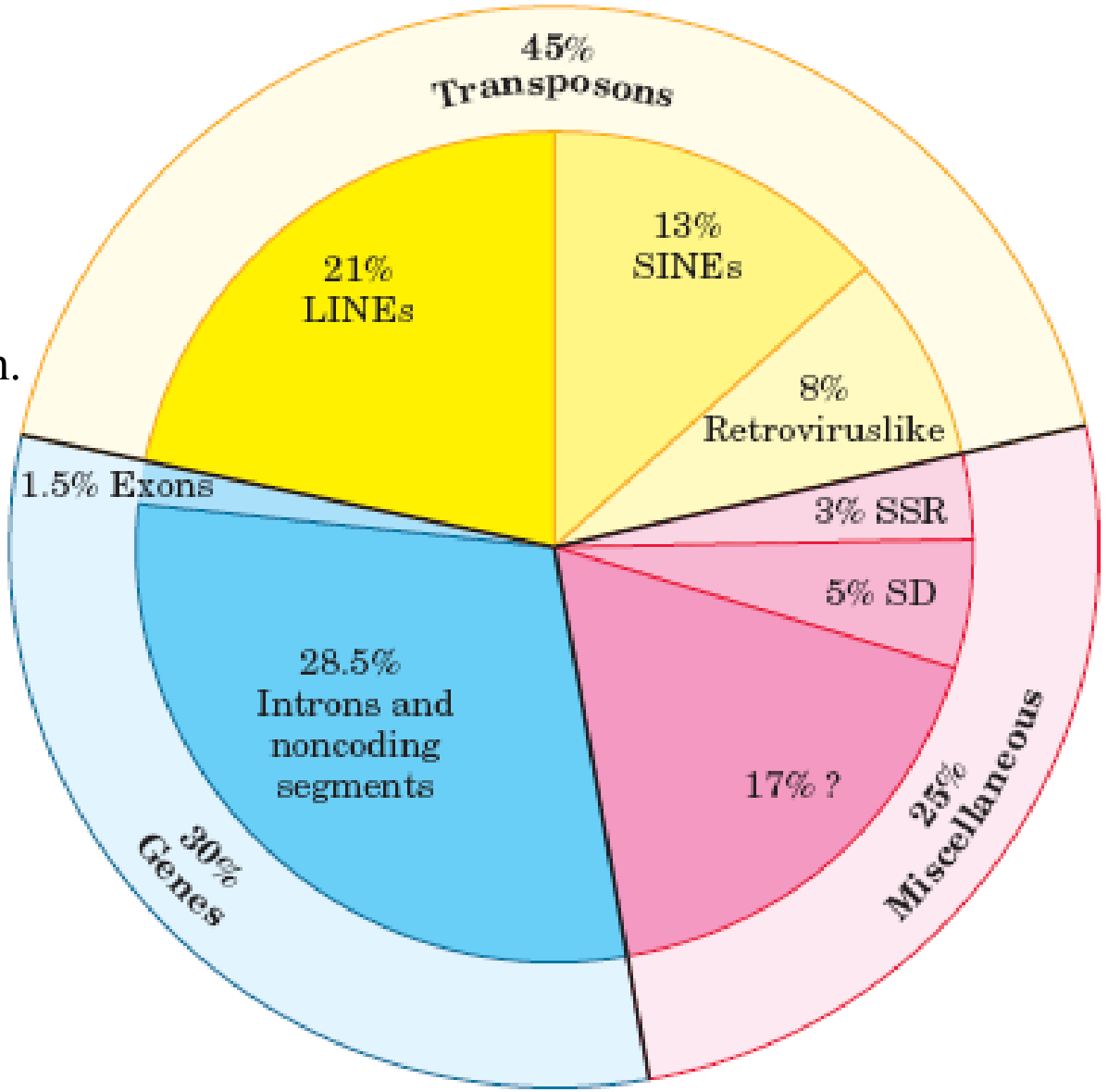
- DNA, that has a base composition (and thus density) sufficiently different from normal DNA that it sediments as a distinct band in caesium chloride density gradients.

Types of sequences in human genome:

SINEs: Short interspersed elements 100-300bp long

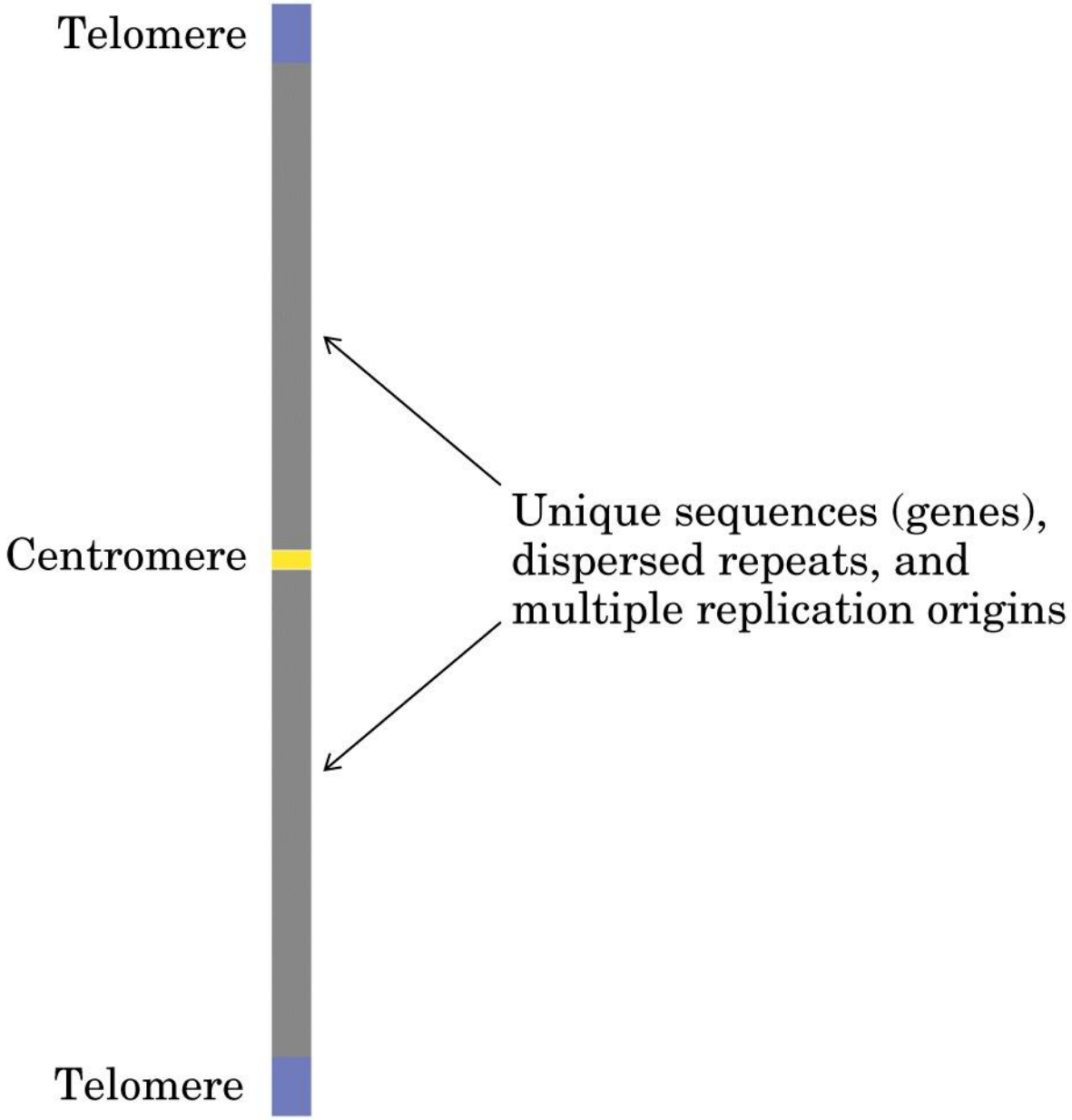
LINEs: Long interspersed elements 6-8kbp, encode a few genes that catalyze transposition.

SSR: simple sequence repeats < 10bp repeated millions times /cell



Centromeres:
a sequence functions during cell division as an attachment point during mitosis,
A=T rich sequence.

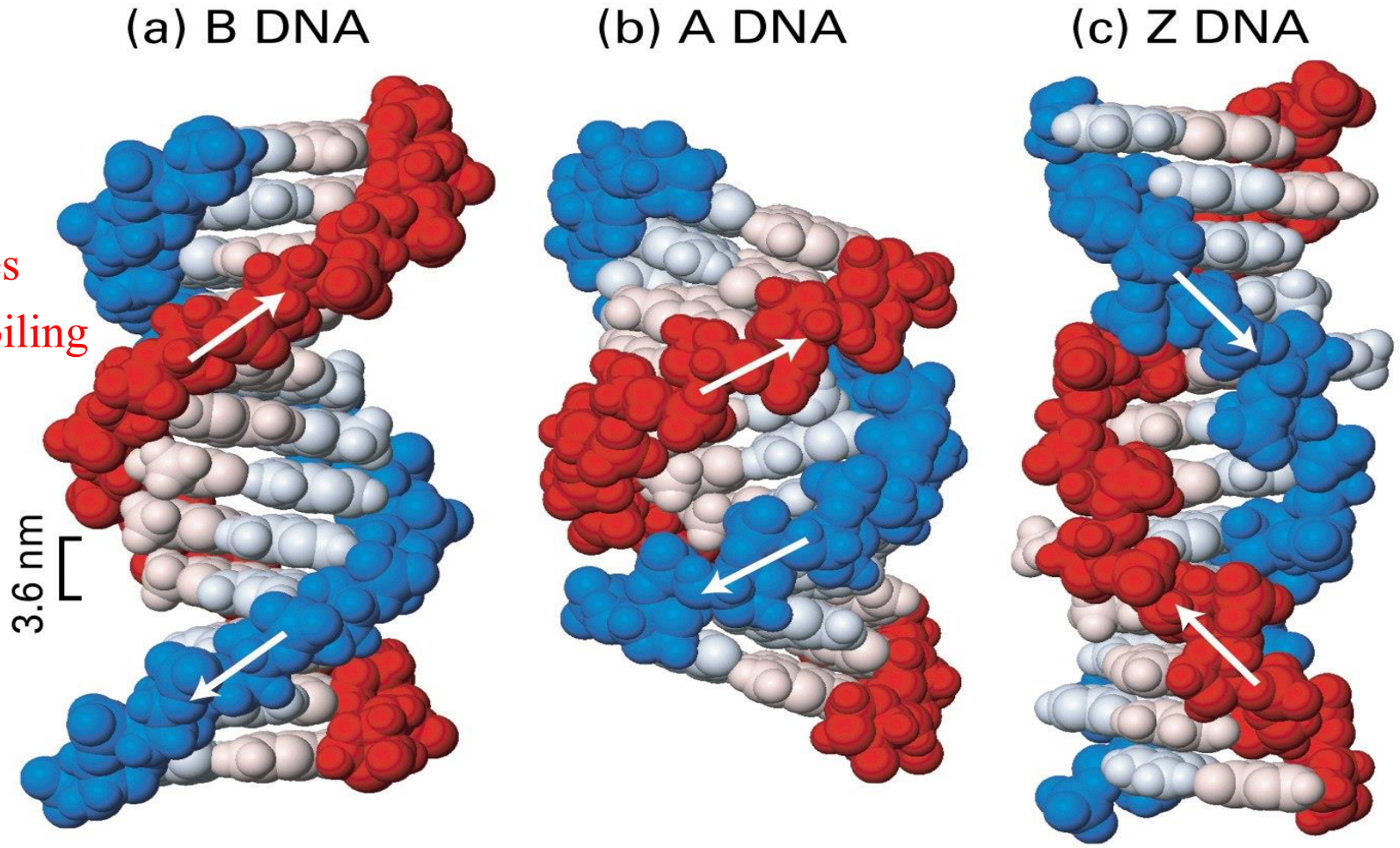
Telomeres:
sequence at the end of chromosomes that help stabilize chromosome



DNA coiled in the form of double helix , with both strands coiling around an axis

	B DNA	A DNA	Z DNA
Helix sense	Right-handed	right	left
Mean bp/turn	10.5	10	12

Further, axis coiling upon itself produces DNA supercoiling

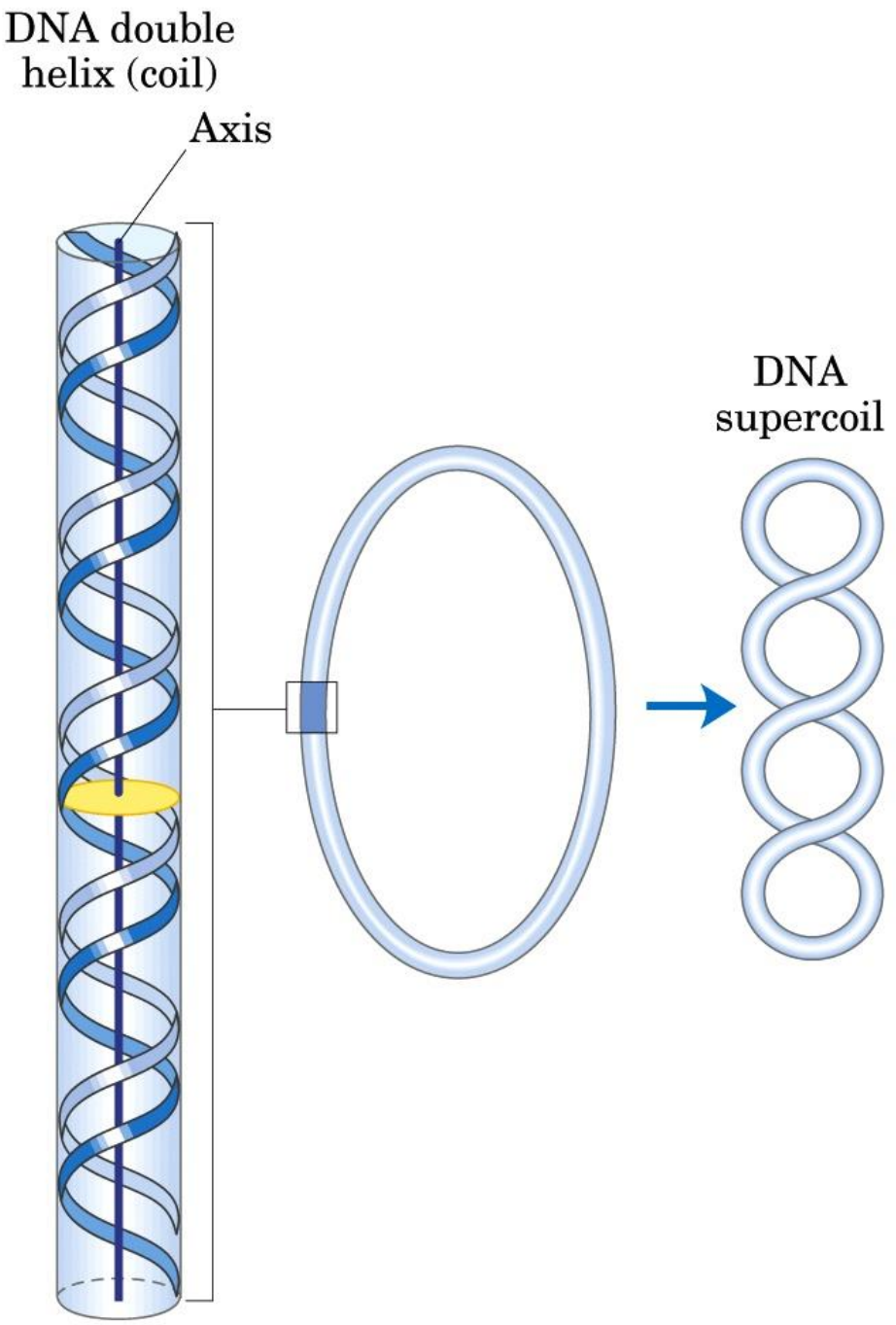


Supercoiling of DNA:

When the axis of DNA double helix is coiled on itself it forms a new helix (superhelix).

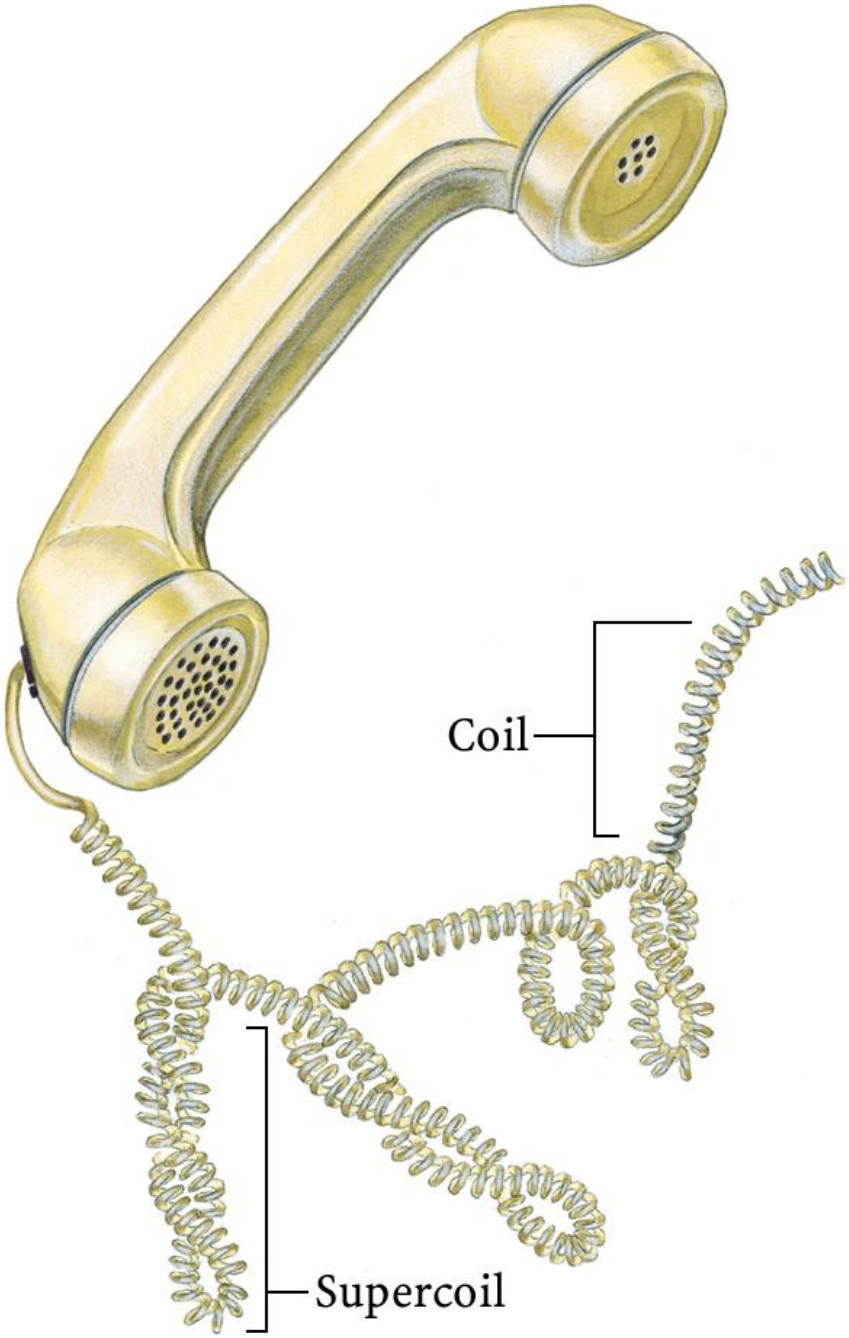
Superhelix = supercoil

The DNA in the **relaxed state**: No net bending of the DNA axis upon itself.



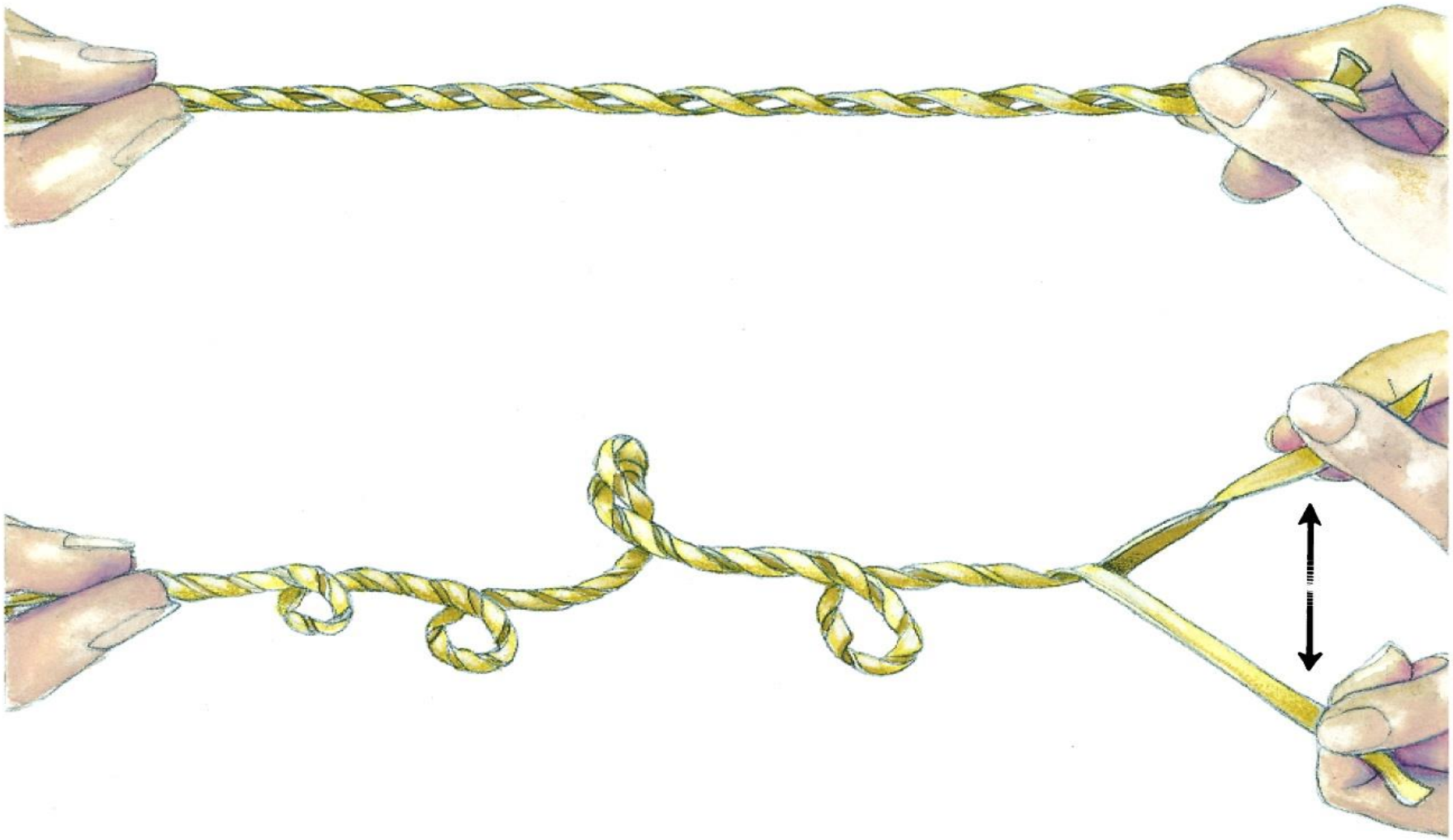
Supercoiling:

coiling of a coil



Supercoiling induced by separating the strands of a helical structure.

Unwinding and subsequent supercoiling occurs during replication, transcription, and protein binding.

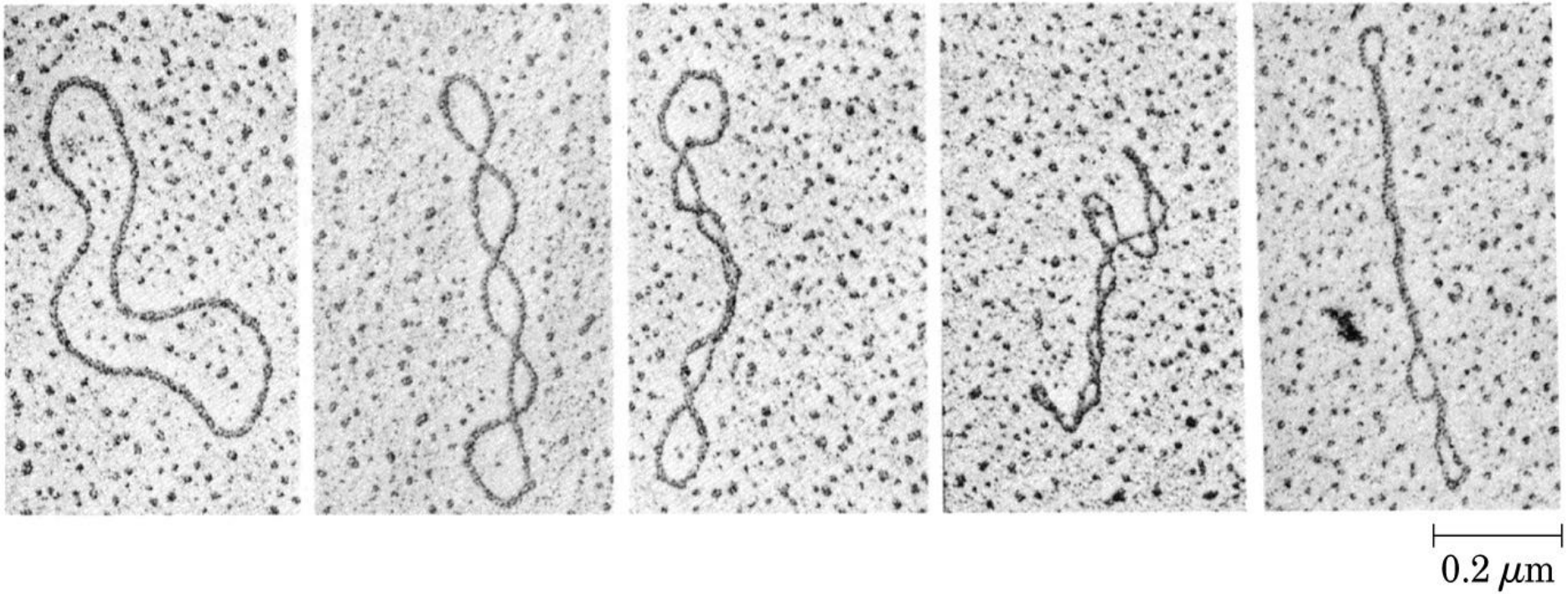


Relaxed and supercoiled plasmid DNA:

Circular DNA remain supercoiled even after extracted and purified.

So, supercoiling is an intrinsic property of DNA tertiary structure.

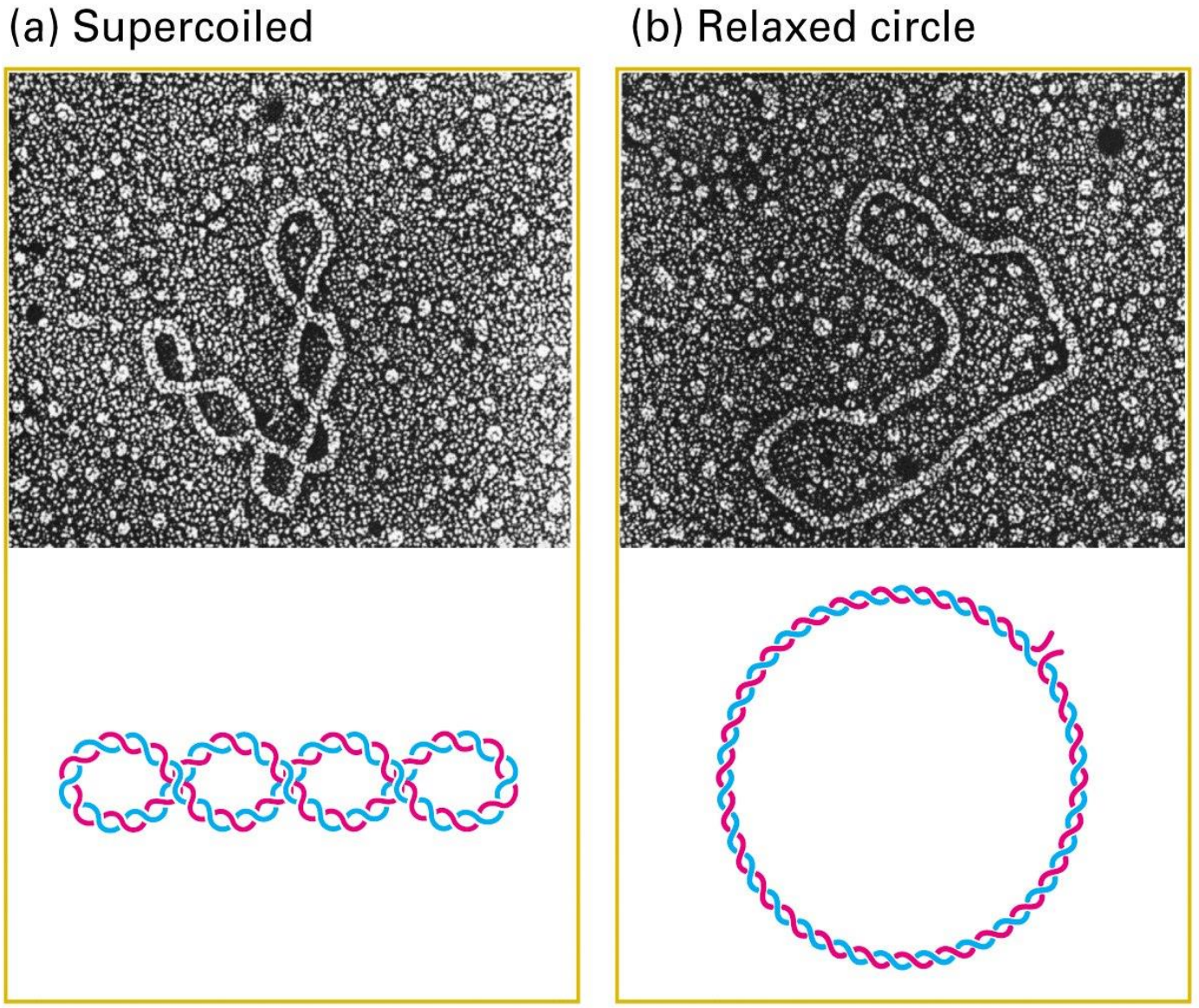
Degree of supercoiling increase →



Many DNA molecules are circular and local unwinding of circular DNA can produce supercoiling

Viral SV40 DNA separated from its protein.

If one strand is nicked the stress is relieved.



Topoisomerases

DNAs that differ only in the linking number (Lk) = **topoisomers.**

Enzymes that underwind/relax DNA = topoisomerases

- The degree of supercoiling in the cell controlled by topoisomerases.

- Advantage: permits DNA to be transiently and locally melted to permit the enzymes of DNA replication and transcription to copy and synthesize new DNA or RNA.

- There are two classes of topisomerase:

  Type 1 topoisomerases

  Type 2 topoisomerases

The best-characterized member of this class in *E. coli*, **Topoisomerase II (DNA Gyrase).**

Topoisomerases are essential enzymes:

topoisomerases → targets for antibiotics and other drugs.

Bacteria can be killed by antibiotics:

novobiocin or nalidixic acid. Both inhibit DNA gyrase. But not eukaryotic topoisomerases.

Eukaryotic topoisomerase inhibitors:

such as doxorubicin and etoposide, are used as chemotherapeutic agents in cancer therapy.

Summary:

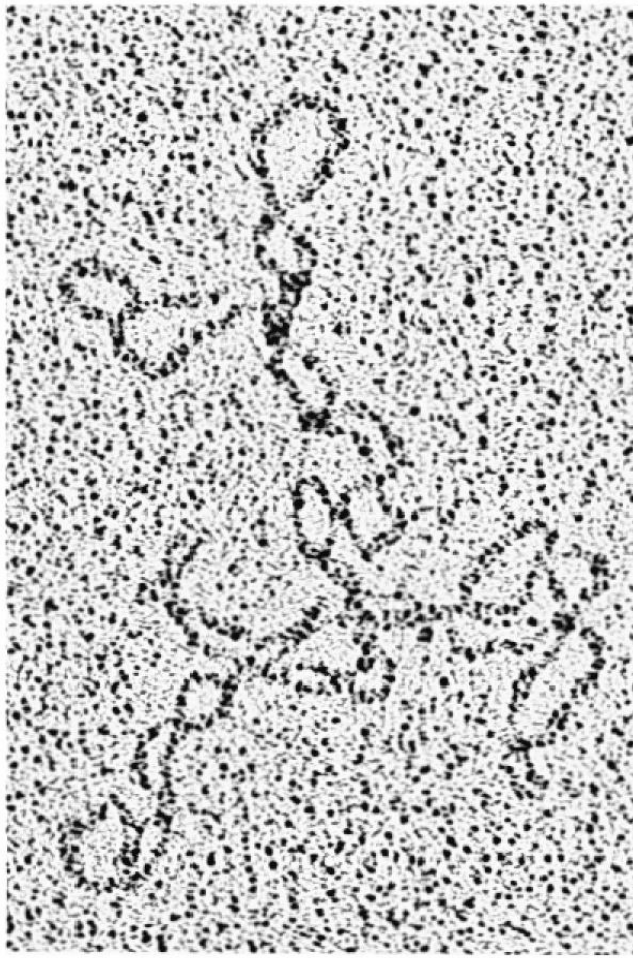
- Unwinding and subsequent supercoiling occurs during replication, transcription, and protein binding.

- Local unwinding of the DNA helix induces stress which is revealed by twisting of the molecule on itself, forming supercoils. This process is regulated by topoisomerases which can remove supercoils.

Forms of supercoiling:

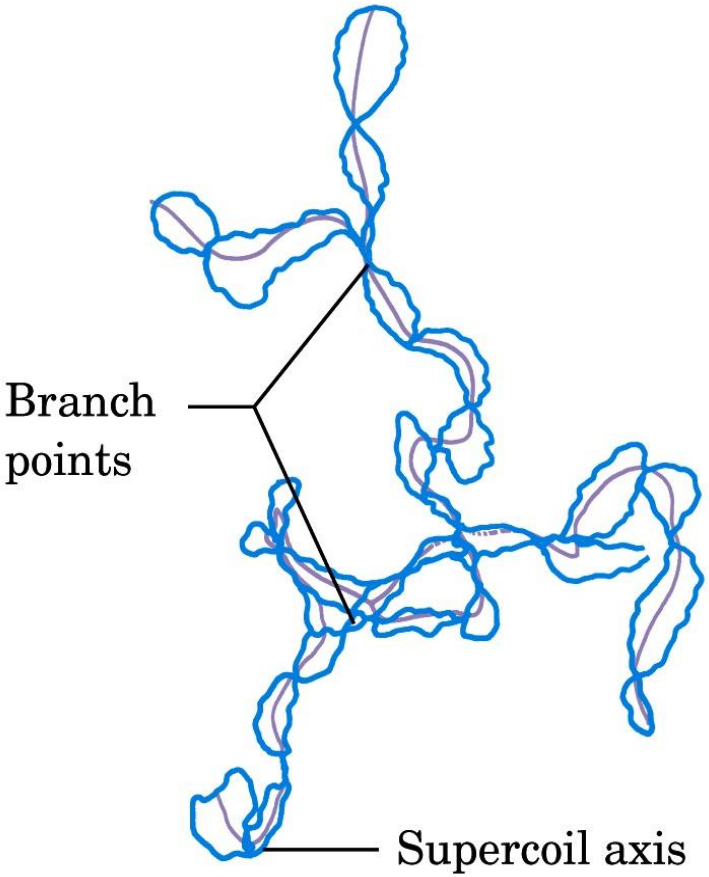
Plectonemic supercoiling : Plectos= twisted, nema= thread

Plectonemic DNA plasmid: extended right-handed coils.



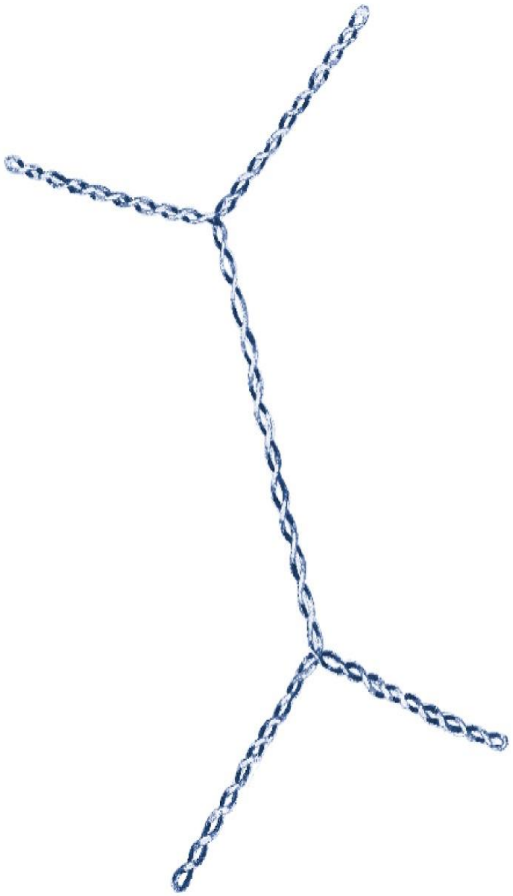
(a)

supercoiled plasmid DNA



(b)

interpretation of the figure



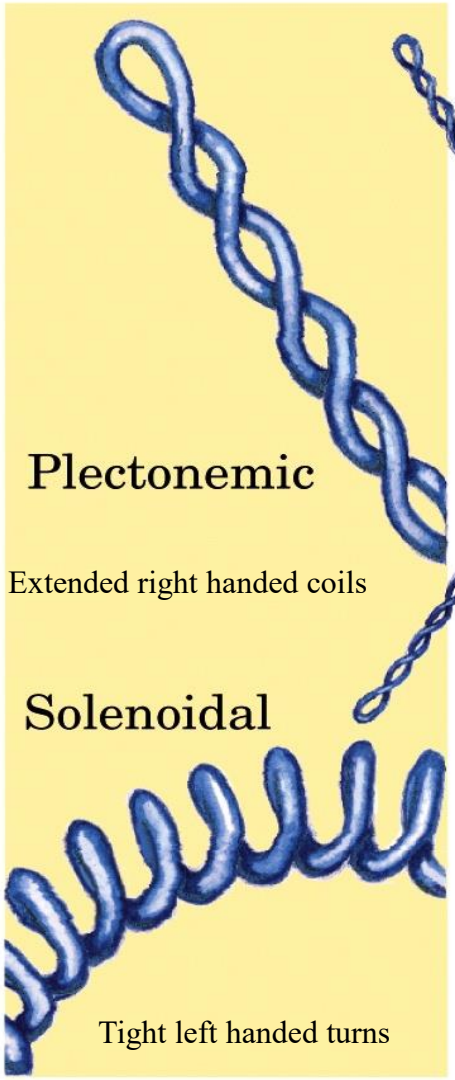
(c)

idealized representation

- **In prokaryotes**, plectonemic supercoils are predominant, because of circular chromosome and small amount of genetic material.
- **In eukaryotes**, both present but solenoidal supercoiling most effective in compacting DNA.

Plectonemic Supercoiling: more stable in solution.

Solenoidal Supercoiling: stabilized and achieved with histones to form a 10nm fiber.



Plectonemic supercoiling does not provide sufficient compaction of DNA in the cell.

Much degree of compaction for the same DNA molecule

(a) (b)

- DNA extracted in isotonic buffer (same salt conc. in cells = 0.15 M KCl) its associated with an equal mass of protein in a highly compacted complex called = nucleosome.

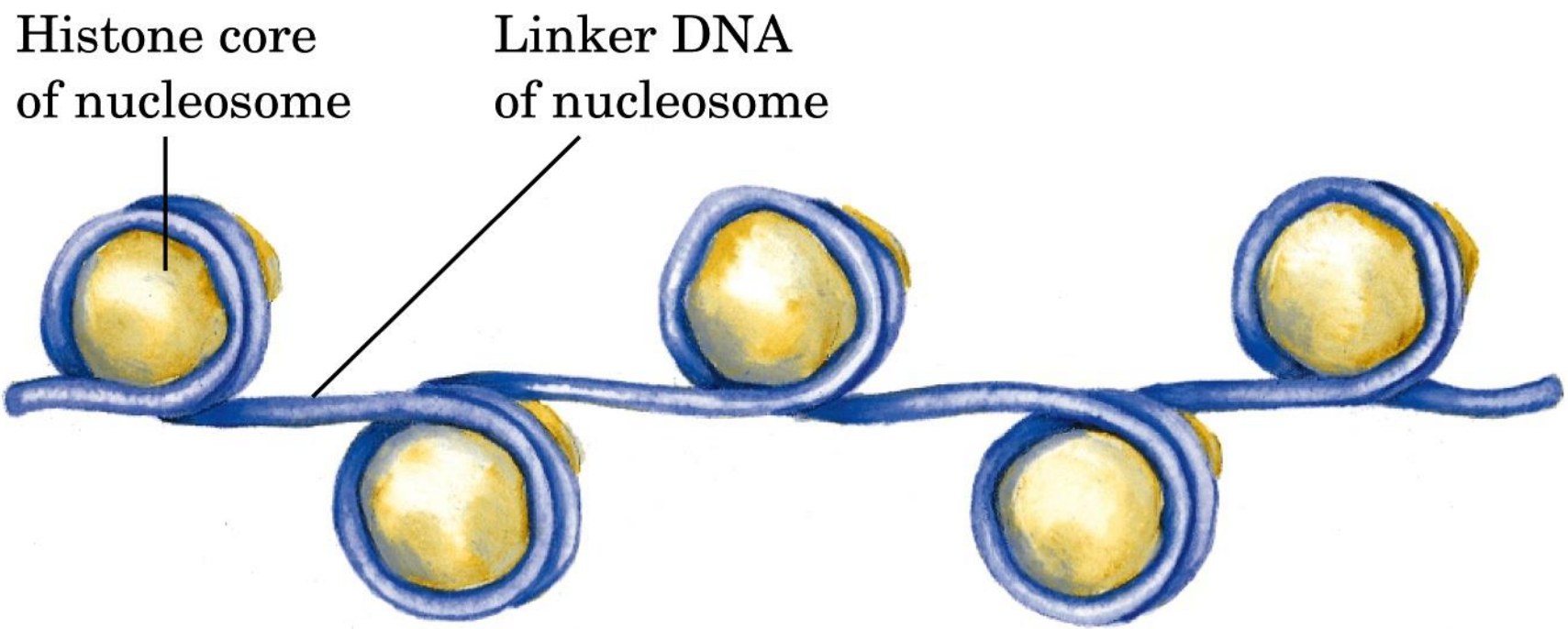
- Five histones H1, H2A, H2B, H3, H4 rich +ve charged basic a.a. interact with -ve charged phosphate groups in DNA.

Beads on a string:

A core nucleosome = 2 copies of 4 histones - H2A, H2B, H3, H4 associate to form an octamer around which **146 bp** of DNA is wound in a **left-handed coil**.

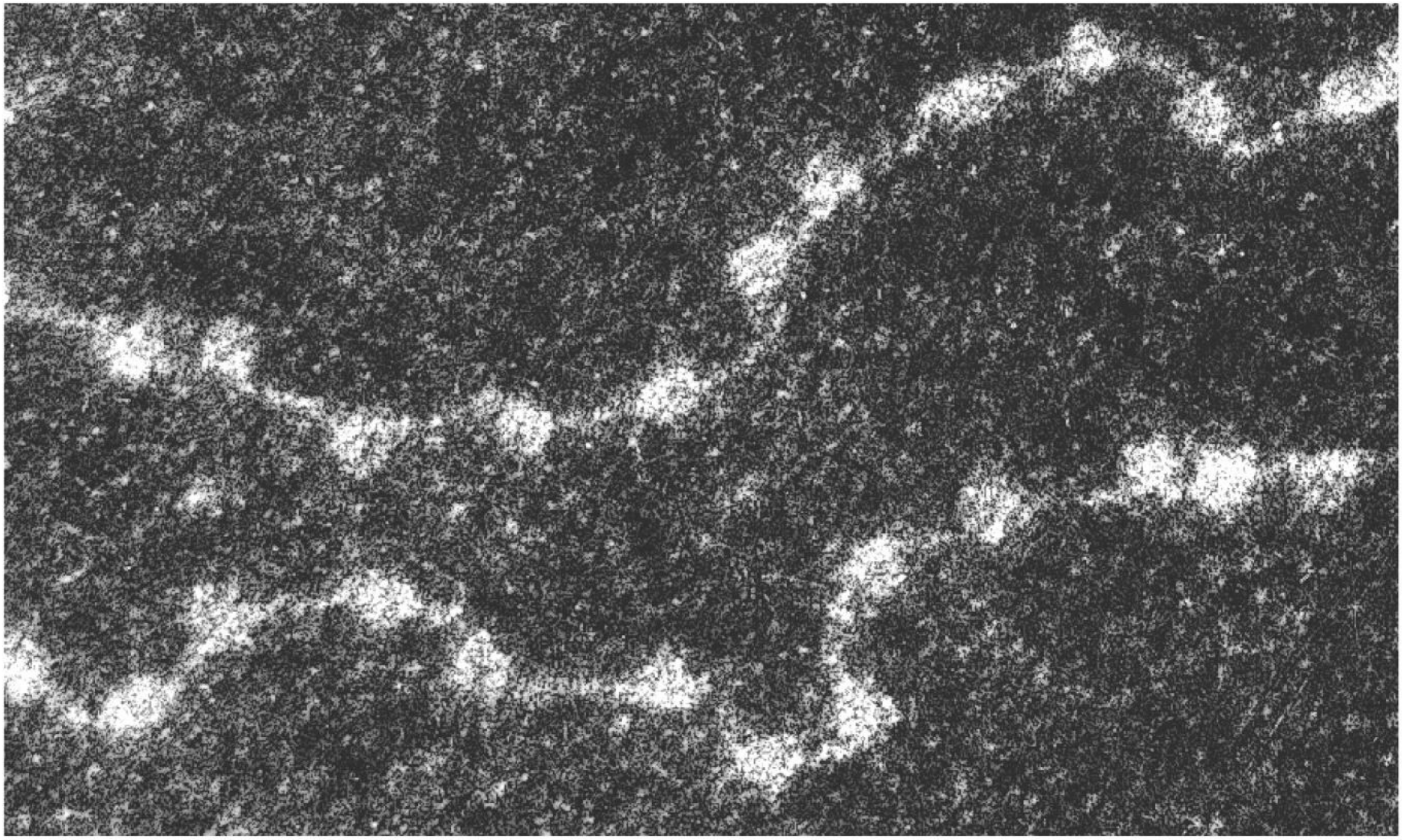
histone proteins rich in +ve charged amino acids (20-30% Arg + Lys) bind to DNA mostly via electrostatic interactions with the phosphodiester backbone of the DNA.

The remainder 200-146= 54 bp = linker DNA



(a)

Electron micrograph :
Nucleosomes, histone complexes bound to DNA



(b)

Nucleosomes two major purposes within the cell nucleus:

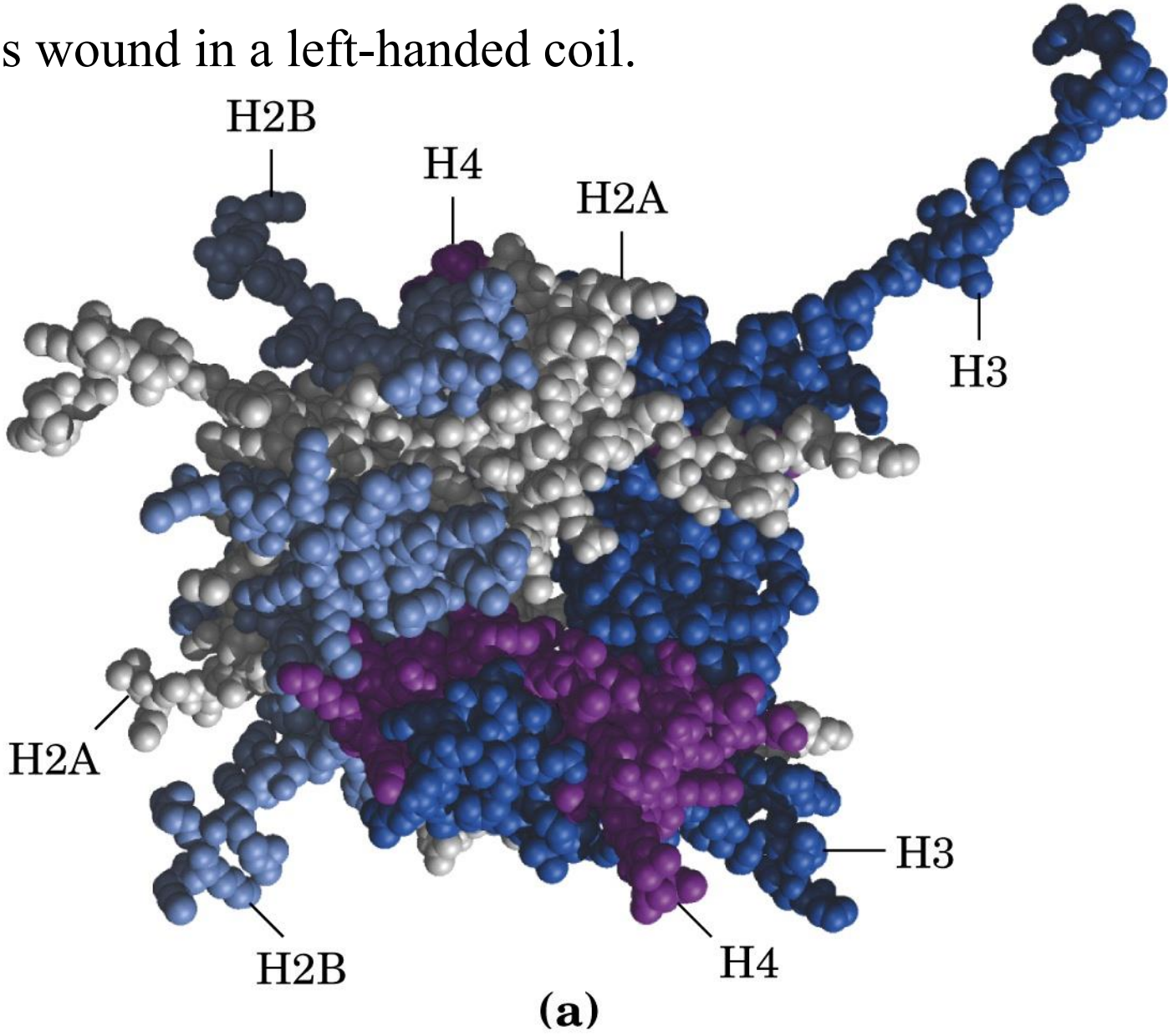
1- provide the level of compaction required to fit dsDNA into the cell nucleus.

2- important in the regulation of transcription by preventing RNA pol from unnecessarily accessing the promoter regions of genes which are not needed by the cell. If the requirements of the cell change, enzymes known as remodeling factors can remove/change the position of the nucleosome to allow access.

A core nucleosome = 2 copies of 4 different histones

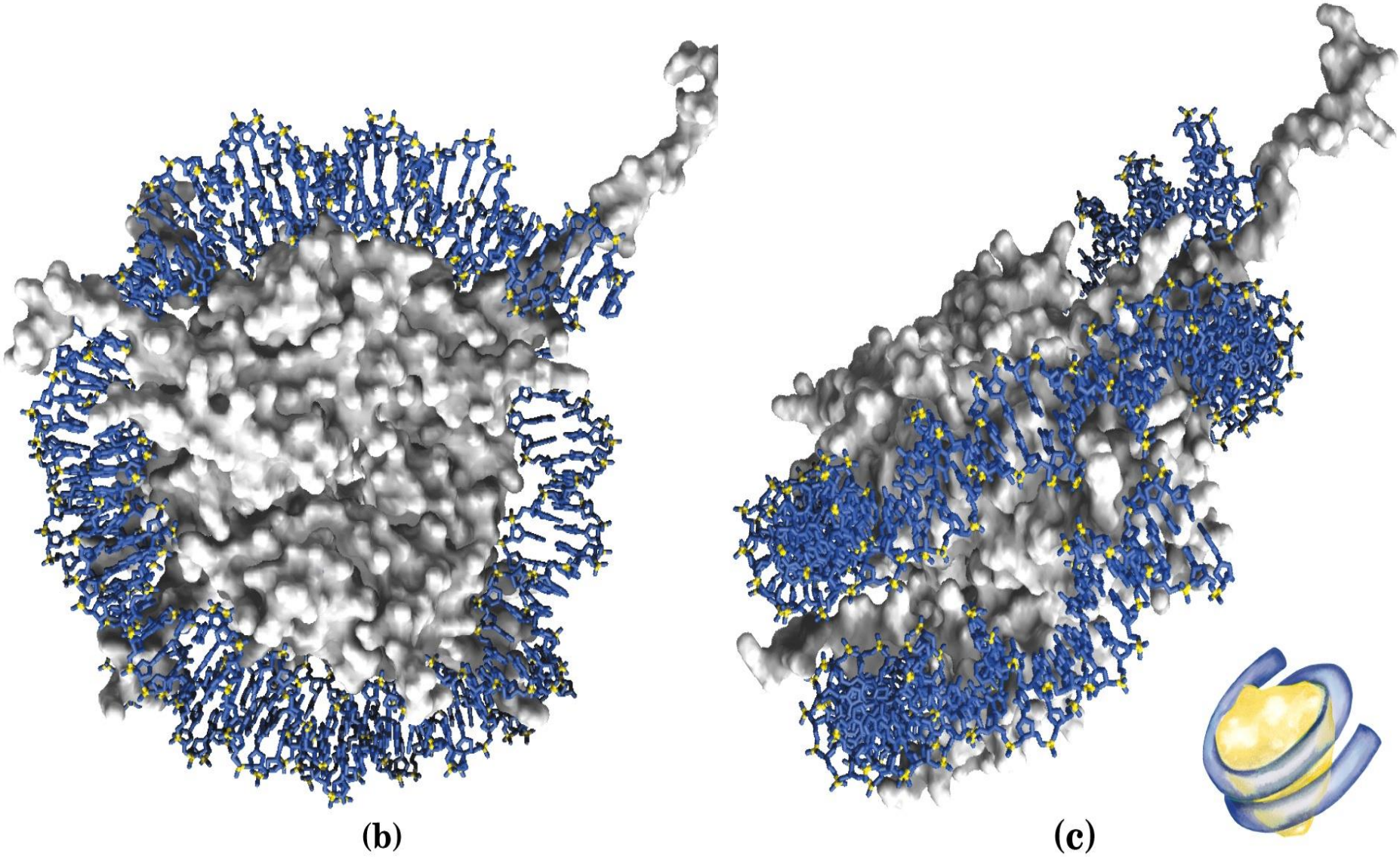
H2A, H2B, H3, H4 associate to form an octamer around which

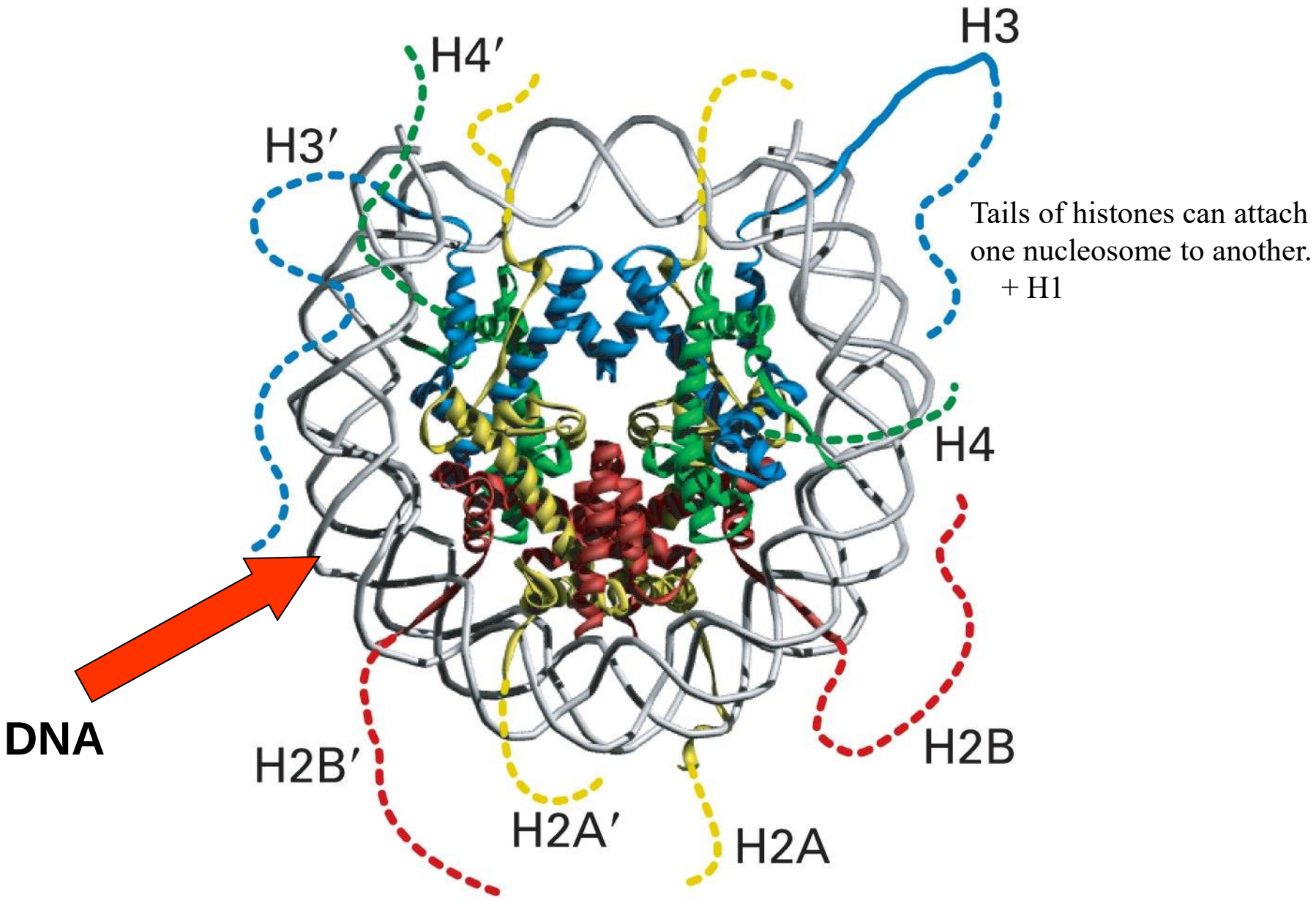
- 146 bp of DNA is wound in a left-handed coil.



DNA wrapped around a nucleosome core : (**1.65 turns**)

The DNA (146 bp) binds in a left-handed solenoidal supercoil.





Tails of histones can attach one nucleosome to another.
+ H1

Nucleosome the building block of chromatin

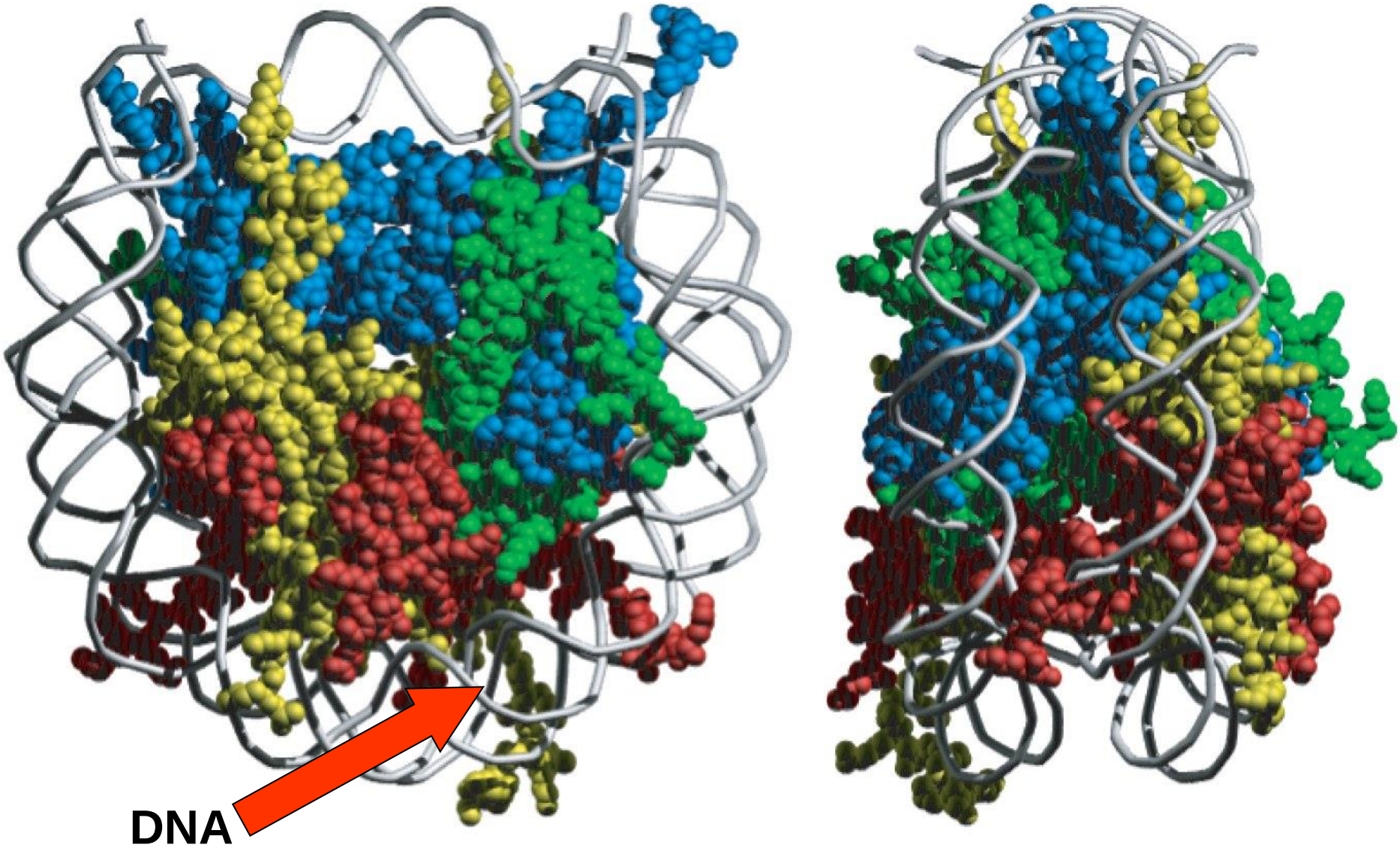


table 24–3

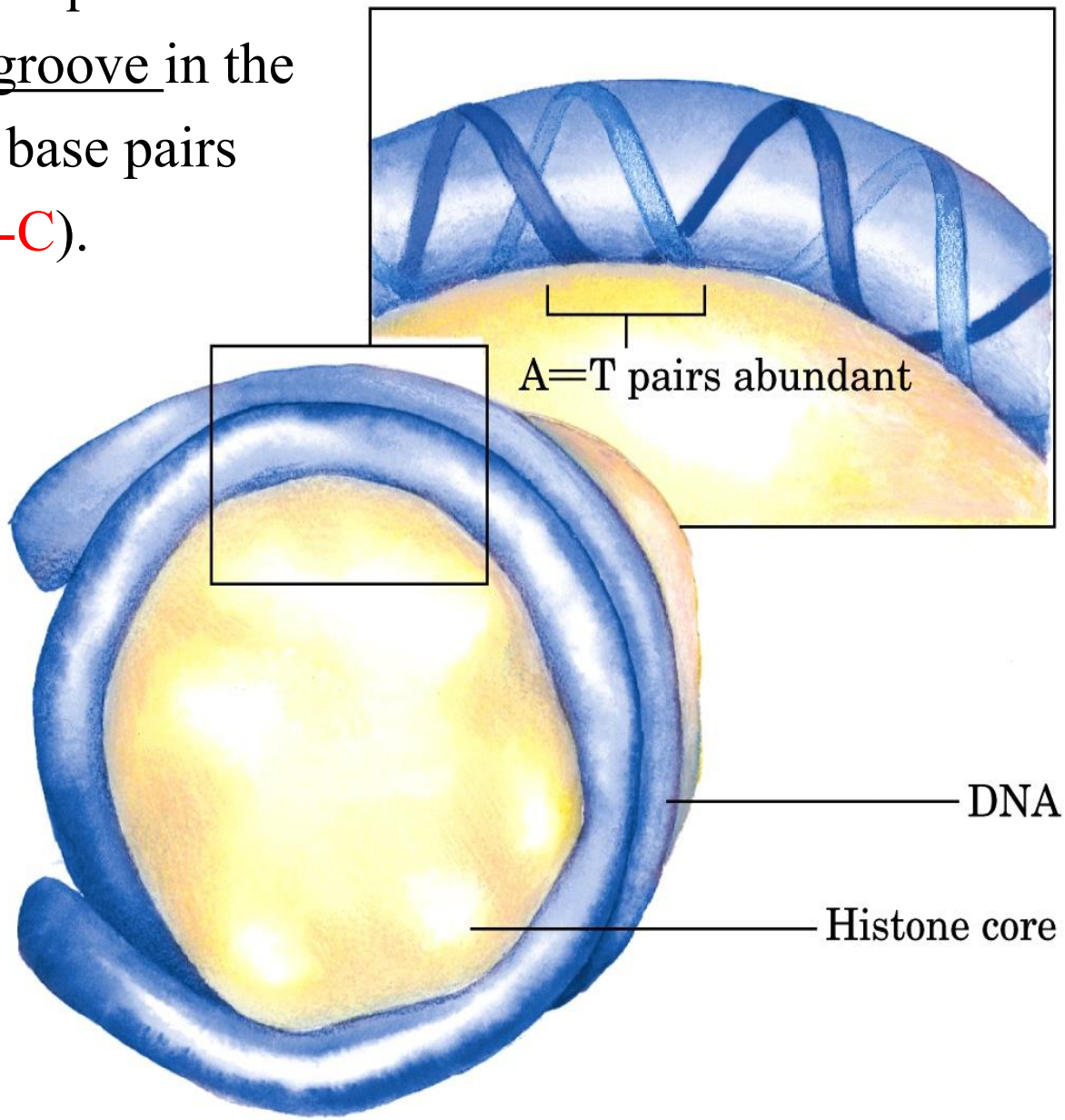
Types and Properties of Histones

Histone	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	1.3
H2A*	13,960	129	10.9	9.3
H2B*	13,774	125	16.0	6.4
H3	15,273	135	9.6	13.3
H4	11,236	102	10.8	13.7

*The sizes of these histones vary somewhat from species to species. The numbers given here are for bovine histones.

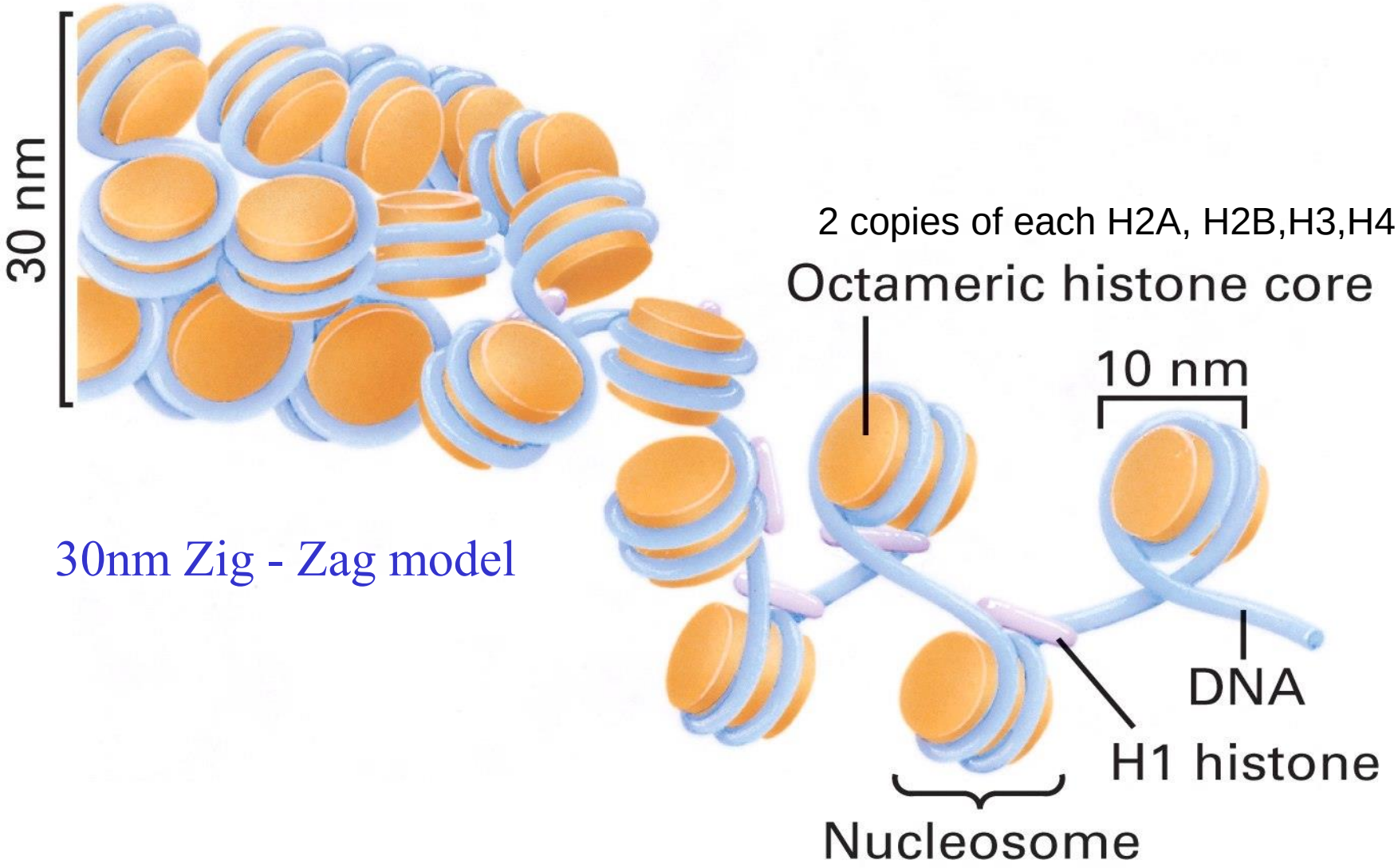
Around one fourth of the a.a in the histones are basic.

Tight wrapping of DNA around the nucleosome's histone core requires compression of the minor groove in the helix at points rich in A=T base pairs (easier to compress than G-C).



Model of the 30nm condensed chromatin :

Each nucleosome associates with one H1 and the fiber coils into a solenoid structure with a diameter of 30nm.



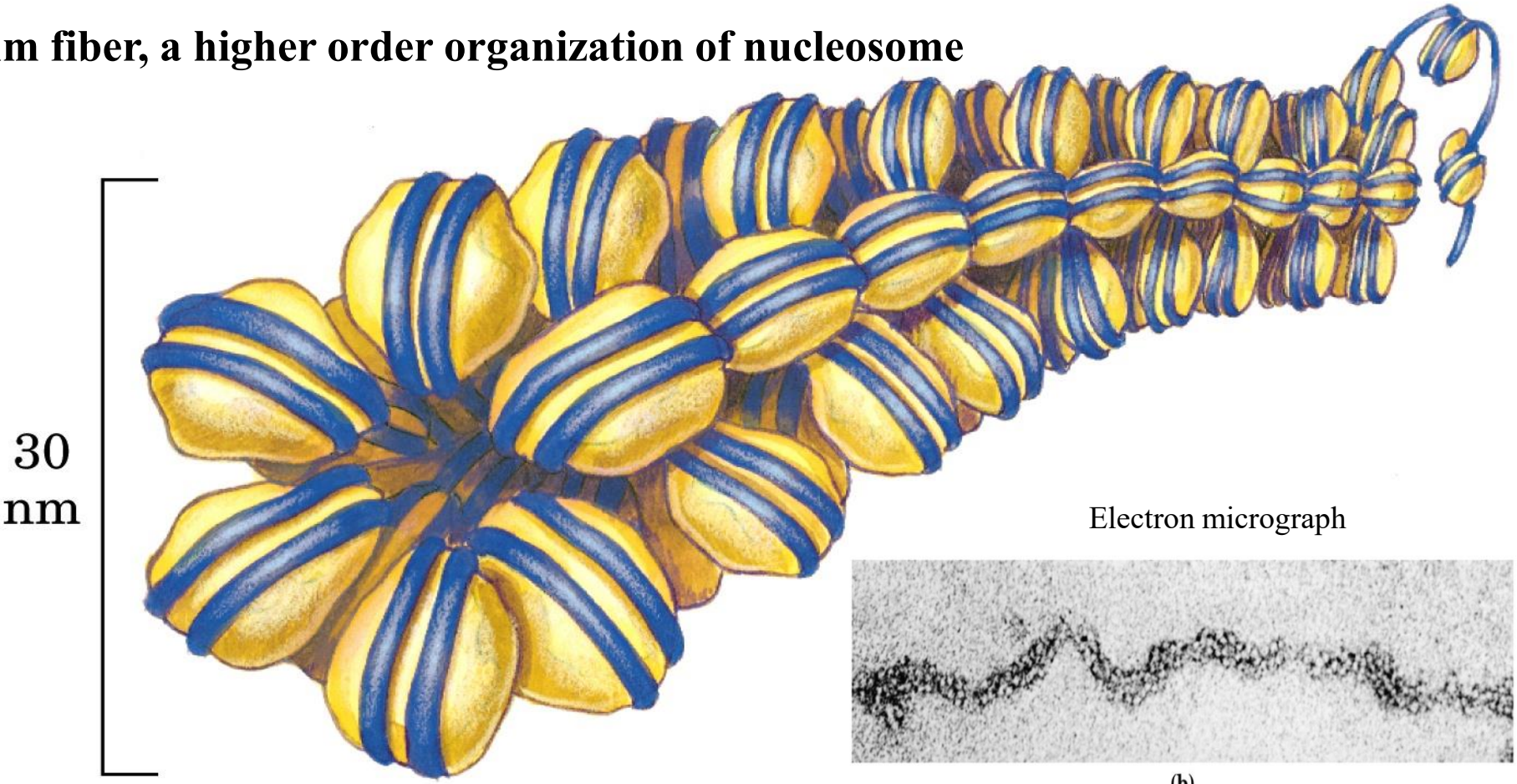
30nm Zig - Zag model

The paradox of the nucleosome structure:

Nucleosome provides a stable structure that protects DNA, on the other hand enzymes e.g DNA or RNA pol cant gain access to DNA template as long as its sequestered in the nucleosome.

In order to be replicated/ transcribed, the nucleosome structure must be partially accessible. The +ve charged amino-termini of H2B, H3, H4 protrude from the core nucleosome and are the targets for acetylation. This "reverses the charge" and disrupts the nucleosome.

30nm fiber, a higher order organization of nucleosome



- Five histones H1, H2A, H2B, H3, H4 rich +ve charged basic a.a. interact with -ve charged phosphate groups in DNA.
- Some modified post-translationally methylation (lysine), acetylation (lysine), phosphorylation (serine), →neutralizing / converting to –ve charge.

* N-termini rich Lysine residues can undergo acetylation / deacetylation.

* The extent of acetylation of histone N-termini ↑, chromatin condensation ↓

  Resistant to digestion by nucleases ↓.

- **Histone Acetylation and Deacetylation**
- In histone acetylation and deacetylation, the histones are acetylated and deacetylated on lysine residues in the N-terminal tail as part of gene regulation.
- "histone acetyltransferase" **(HAT)** or "histone deacetylase" **(HDAC**). The source of the acetyl group in histone acetylation = Acetyl-CoA, The acceptor of acetyl group in histone deacetylation is CoA.
- Acetylation brings a –ve charge, neutralise the +ve charge on the histones and decreases the interaction of N termini of histones with –ve charged phosphate groups of DNA. **the condensed chromatin is transformed into a more relaxed structure** which is associated with greater levels of gene transcription. This relaxation reversed by HDAC activity.
- Relaxed, transcriptionally active DNA =**euchromatin**.
- condensed (tightly packed) DNA =**heterochromatin**..

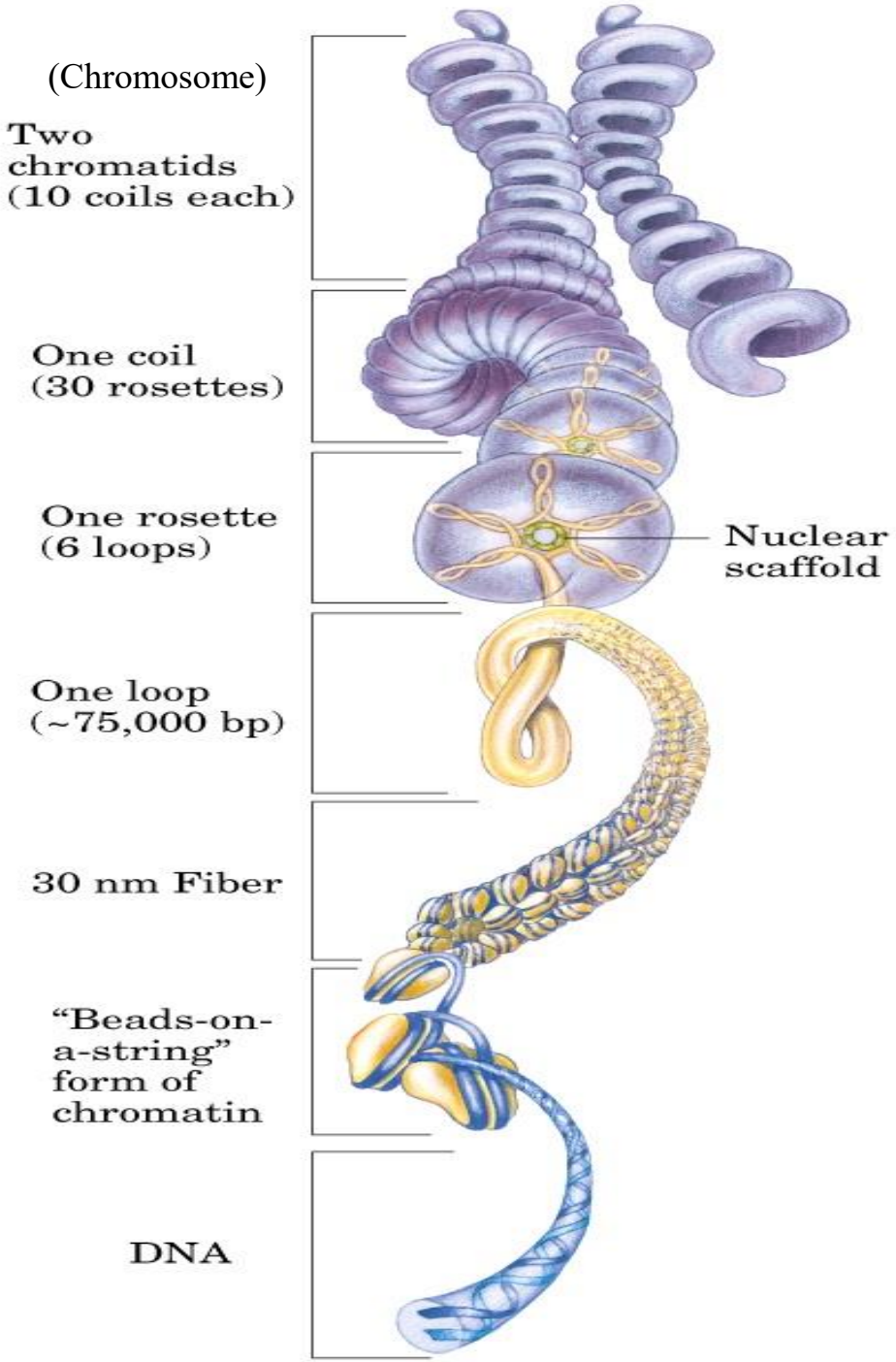
Wrapping DNA around a nucleosome core compacts the DNA length about **7 fold**.

However, the overall compaction in a chromosome is **>10,000 folds**

Nucleosome cores organized into 30nm fiber requiring one H1 per nucleosome provide **100 fold** compaction.

Higher level of folding =

Nuclear Scaffold containing H1, topoisomerase II, and SMC proteins.


(Chromosome)
Two chromatids (10 coils each)
One coil (30 rosettes)
One rosette (6 loops)
Nuclear scaffold
One loop (~75,000 bp)
30 nm Fiber
"Beads-on-a-string" form of chromatin
DNA

Maintenance of condensed chromosome structure:

Chromatin proteins: 3 classes

Histones, topoisomerase, SMC

SMC proteins (Structural Maintenance of Chromosomes)

- a large family of ATPases participate in many aspects of higher-order chromosome organization and dynamics.

- Present in all organisms bacteria→ human.

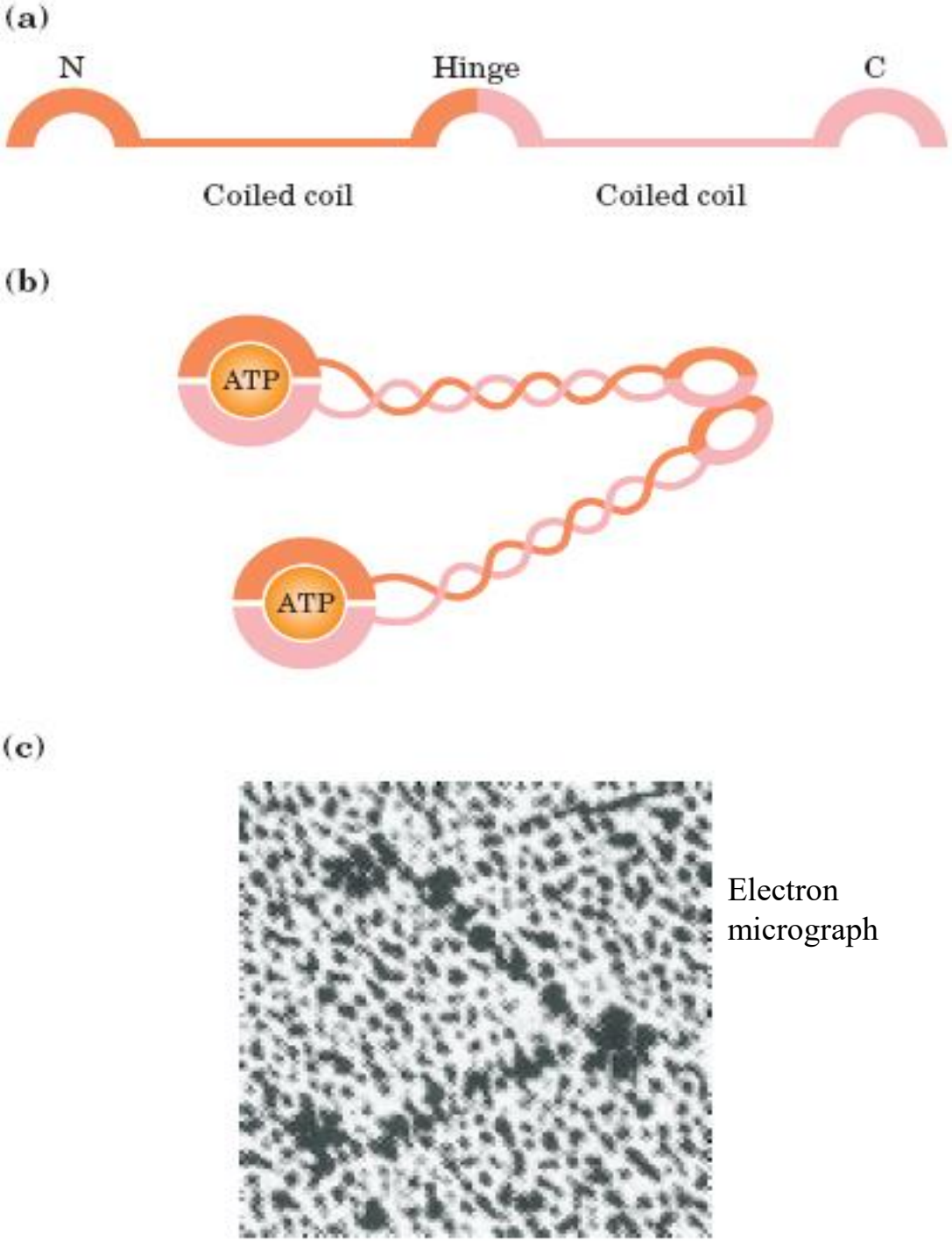
- Eukaryotes have at least six SMC proteins in individual organisms, and they form three distinct heterodimers with specialized functions.

Structure of SMC :

5 domains amino & carboxyl domains, hinge, coiled coils.

Each polypeptide folded →two coiled coils domains wrap around each other the N & C domains form a complete ATP binding site.

Two of these domains linked at the hinge region to form **dimeric V-shaped molecule**



Electron micrograph

SMC :

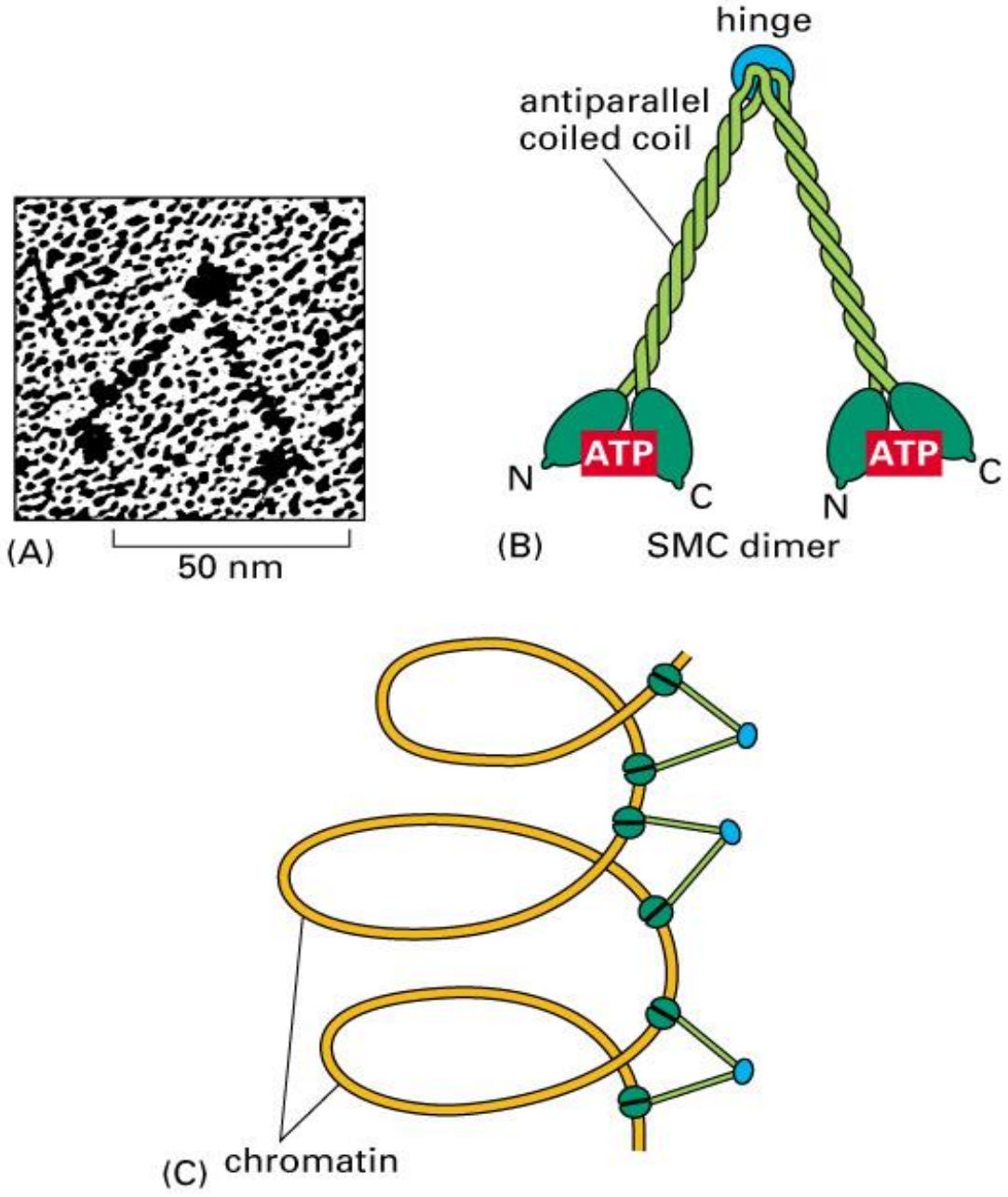


Figure 4–56. Molecular Biology of the Cell, 4th Edition.

- **Solenoidal** supercoiling achieved with histones →coiled into 30nm fiber → coiled upon itself numerous times more.

1) DNA packaging greatly increased during **nuclear division** (mitosis / meiosis) where DNA must be compacted and segregated to daughter cells.

2) Supercoiling required for **DNA / RNA synthesis**. Because DNA must be unwound for DNA/RNA pol action, supercoils will result.

   Topoisomerases such as DNA gyrase (Type II Topoisomerase) relieve some of the stress during DNA/RNA synthesis.

E. coli cells showing nucleoid:
DNA stained with a dye
some cells with multiple nucleoids.
Some undergone cell divisions others not.

Bacterial cell division cycle can be as short as 15min, in eukaryotes hours- months.

Bacterial DNA is also compacted in nucleoid
Histone like proteins HU