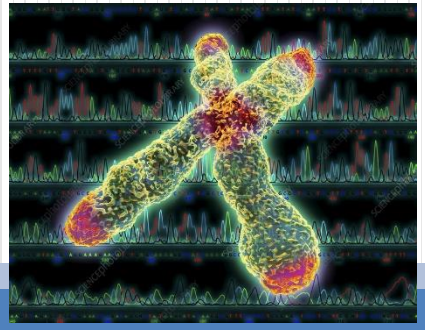
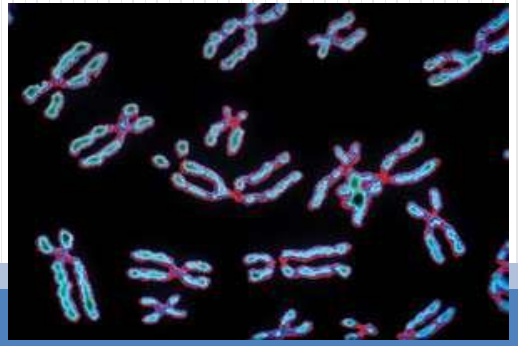




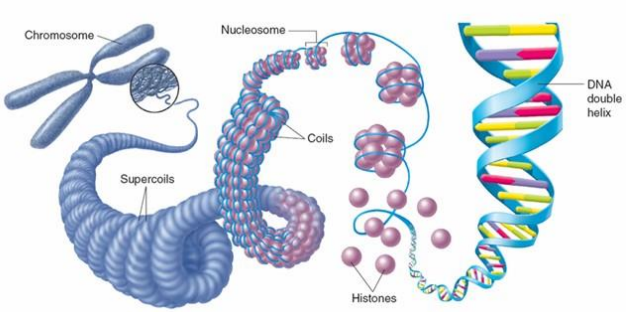
Chromosomes
DNA and chromosome packaging

Lecture 9
SLE254 Genetics and Genomics

Concepts of Genetics (12th ed)

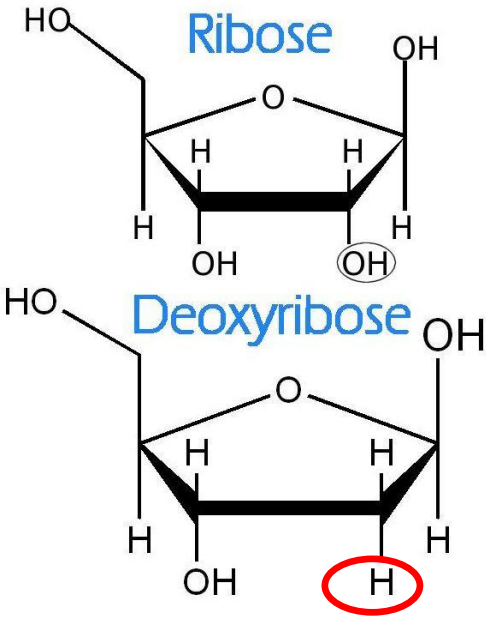
Ch10: pp 251-274

Ch12: pp 302-319



The structure of nucleic acids

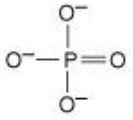
- Nucleic acids are composed of strings of nucleotides linked together. A nucleotide consists of :
  - A phosphate group (Backbone PO_4^{3-})
  - A pentose sugar *either*
    - Deoxyribose (DNA) (2-Deoxyribose)
    - Ribose (RNA)
  - A nitrogen-containing base
    - Adenine
    - Guanine
    - Cytosine
    - Thymine (DNA only)
    - Uracil (RNA only)



The structure of nucleic acids

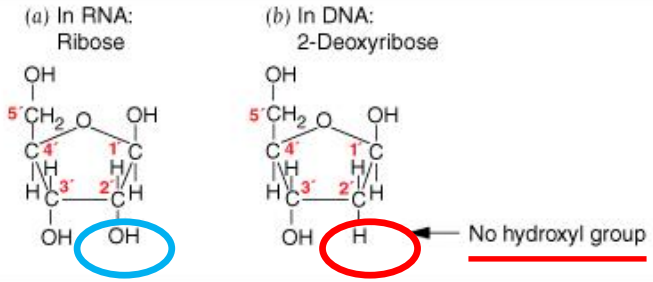
Backbone

(1) A phosphate group:



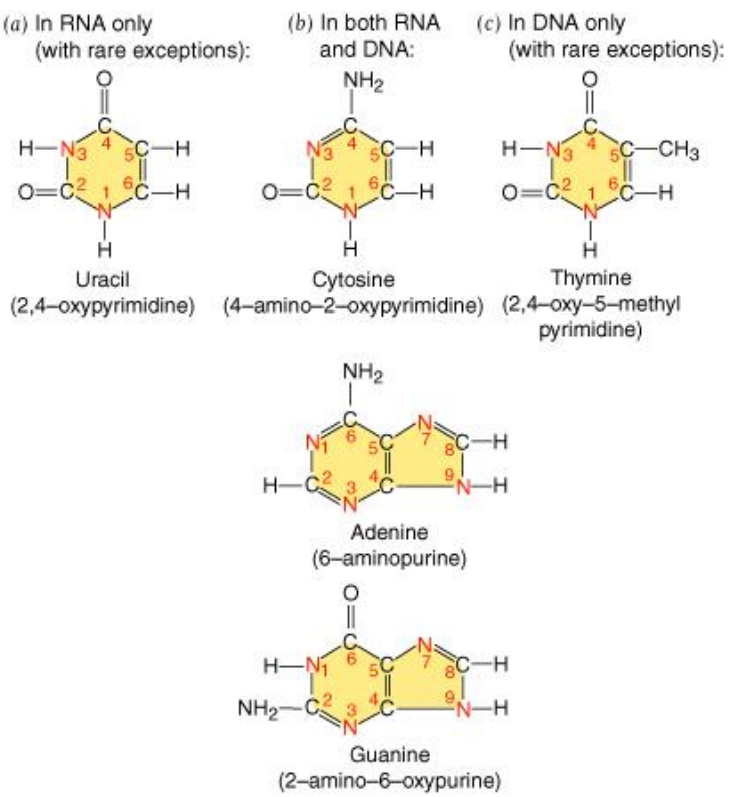
Pentose sugar

(2) A five-carbon sugar or pentose:



Nitrogenous base

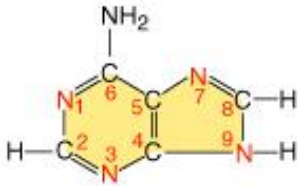
(3) A cyclic, nitrogen-containing base:



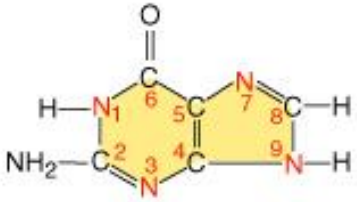
You wont need to draw the structure- you will need to know that hydroxl is situated at 2' carbon atom (ribose) and the phosphate attaches to the 5' atom

← Pyrimidines (Single ring)

← Purines (Double ring)



Adenine
(6–aminopurine)



Guanine
(2–amino–6–oxypurine)

Adenine – Cytosine – Guanine – Thymine/Uracil

Purine	Pyrimidine	Purine	Pyrimidine
Double	**Single**	**Double**	**Single**

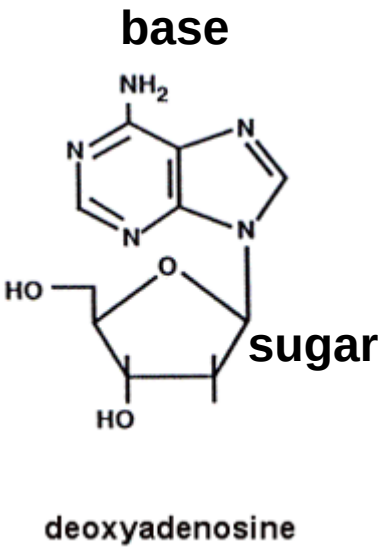
Cytosine
(4–amino–2–oxypyrimidine)

Thymine
(2,4–oxy–5–methyl
pyrimidine)

Uracil
(2,4–oxypyrimidine)

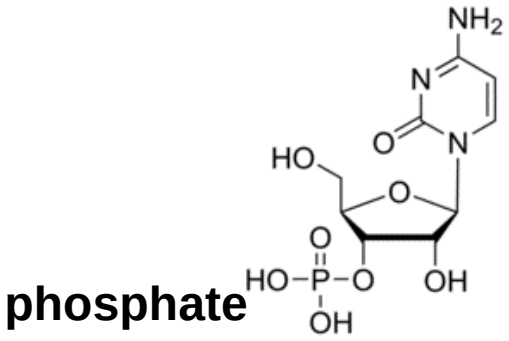
Nucleotides and nucleosides

- A **nucleoside is a base plus sugar- *NO phosphate***
  - E.g. cytosine (the base) plus deoxyribose (the sugar) = **deoxycytidine** (the nucleoside)
  - E.g. Adenine (the base) plus deoxyribose (the sugar) = **deoxyadenosine** (the nucleoside)

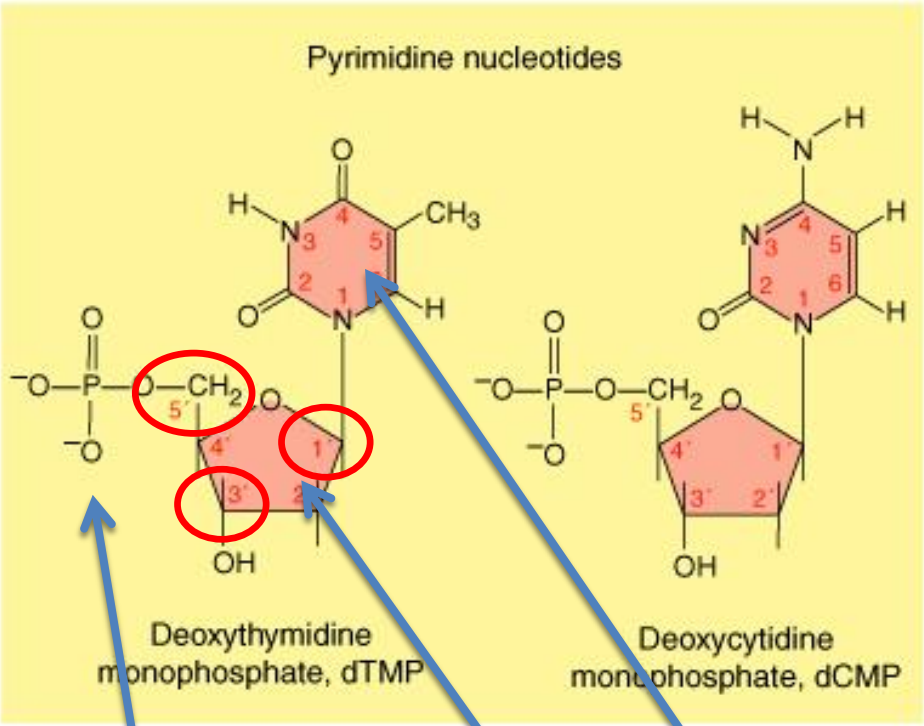


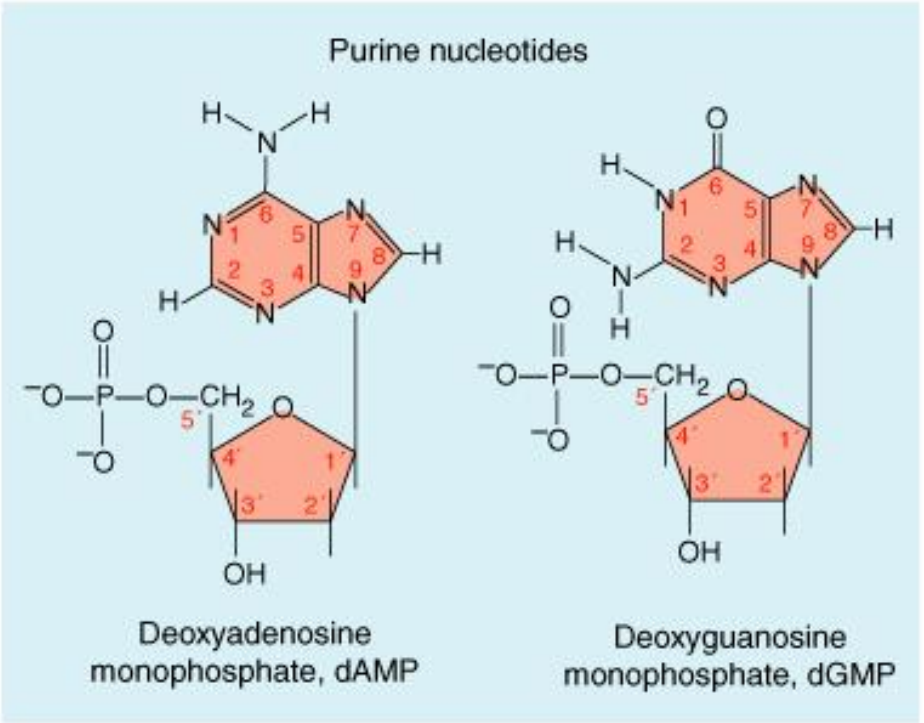
deoxyadenosine

- A **nucleotide is a *nucleoside* plus a phosphate**
  - E.g. Cytidine 3'-phosphate



Nucleotides: learn the attachment sites





Phosphate Backbone

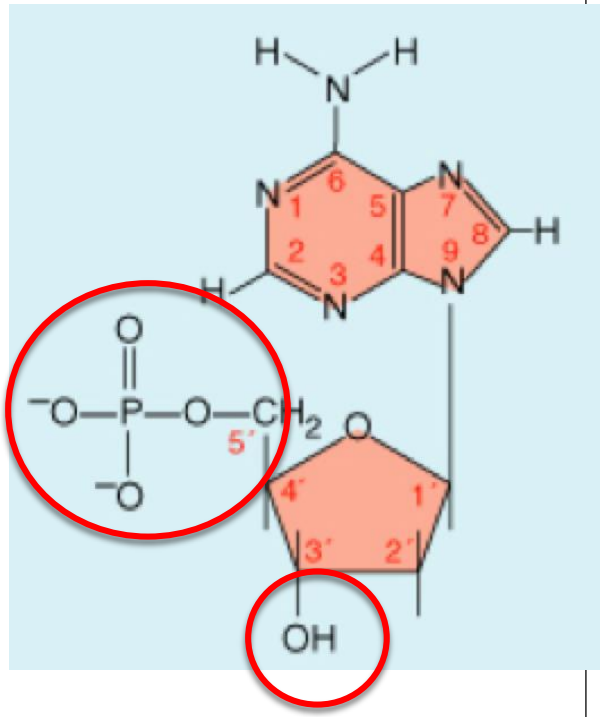
Pentose sugar, deoxyribose

Nitrogen containing 'Base'

These are monophosphate nucleotides, but can have di (2X) and tri (3X) phosphates. Example, ATP (adenosine triphosphate).

Polynucleotides are DIRECTIONAL

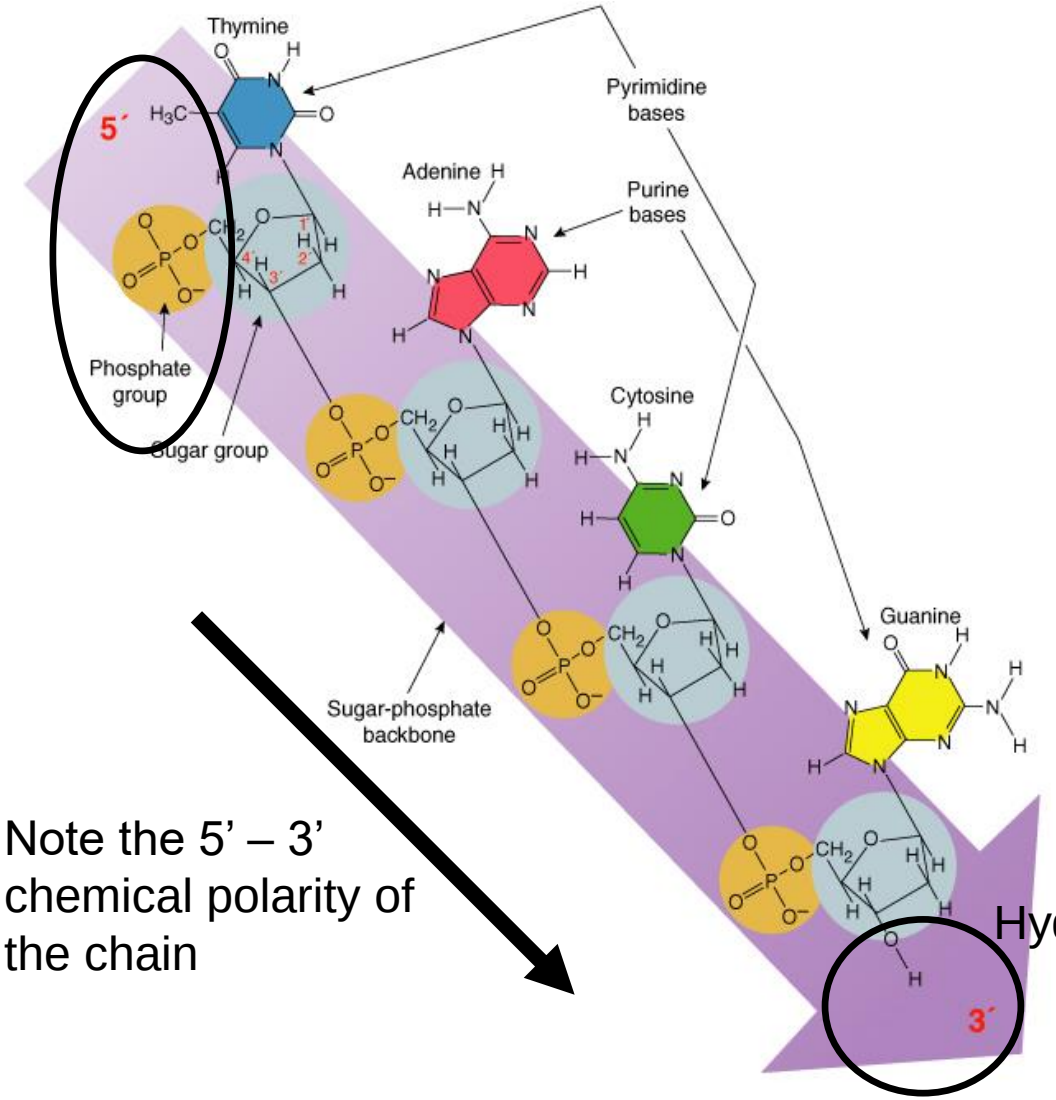
- Nucleotides are joined together to form a **polynucleotide chain**
- Polynucleotide chains have slightly different structures at either end
  - Phosphate group at 5’ end
  - OH group at 3’ end



- DNA is a double helix of two polynucleotides held together by hydrogen bonds
  - The two strands run in **different** directions:

Definitions: 3’ and 5’ directions

Polynucleotides are directional

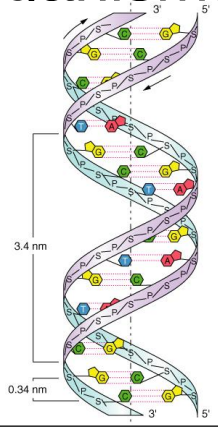


Note the 5’ – 3’ chemical polarity of the chain

- Is this polynucleotide chain DNA or RNA?
- Is it single or double stranded?

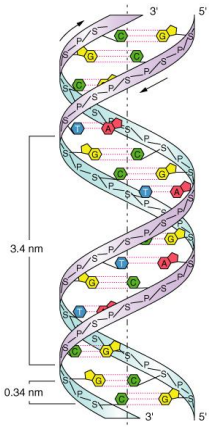
Specific ratios of bases in DNA

- Erwin Chargaff (1952) analysed the composition of DNA from many different organisms and found:
  - Concentration of T(Thymine) = concentration of A (Adenine)
  - Concentration of C (Cytosine) = concentration of G (Guanine)'
  - Therefore, concluded that they **exist in pairs** (base pairs) **and thus always equal in concentration**
  - But [T+A]/[C+G] ratio varied widely in different organisms. Humans have a more [T+A] in DNA (60%)
  - WHY in human? We don't really know



DNA has a double helix structure

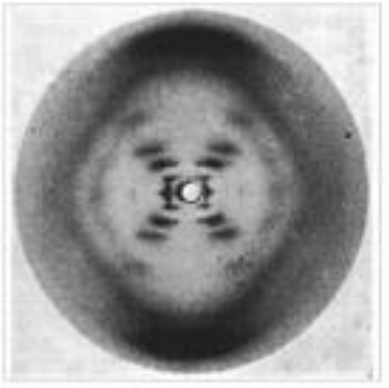
- The structure of DNA was deduced from **X-ray diffraction patterns** by Watson and Crick (1953)
- It is a **right handed double helix (clockwise)**
- The two polynucleotide chains are held together by hydrogen bonding between the bases and hydrophobic interactions
- The base pairing is very specific: A with T and G with C



How did Watson and Crick work out the structure of DNA?

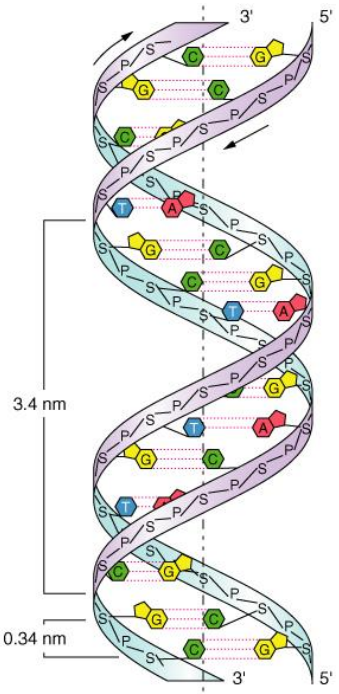
- They used X-ray data from **Rosalind Franklin** and **Maurice Wilkins** (Nobel prize to Watson, Crick and Wilkins; 1962)
- This suggested a double helical arrangement
- The data indicated that DNA was a highly ordered two stranded structure
- A repeating substructure every 0.34 nanometers (base stack), 'between each base'

"Photo 51"

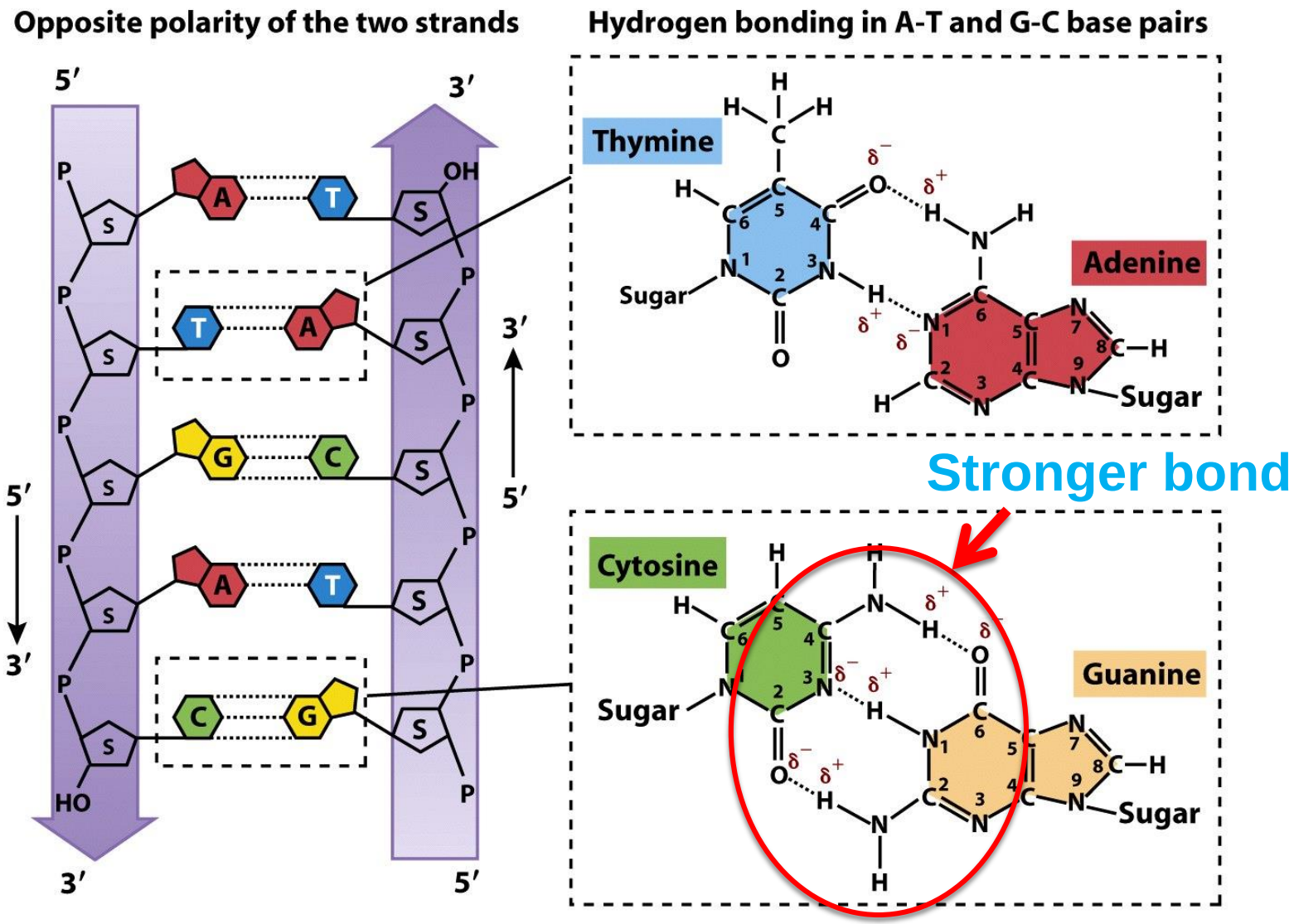


How did Watson and Crick work out the structure of DNA?

- **Watson** realized Chargaff's data indicated a very specific relationship between the bases
- **Crick** proposed that the base pairing could be accommodated in a double helix with **antiparallel** strands and internal bases



Relationship between the bases



Double helix

- DNA is a double helix. The two strands are held together by complementary base pairing.

- In DNA the amount of A= T and the amount of G=C.
- This is because these bases pair specifically together.
- Turn every 10 bases, with two groove sizes. Grove size important for transcription factors

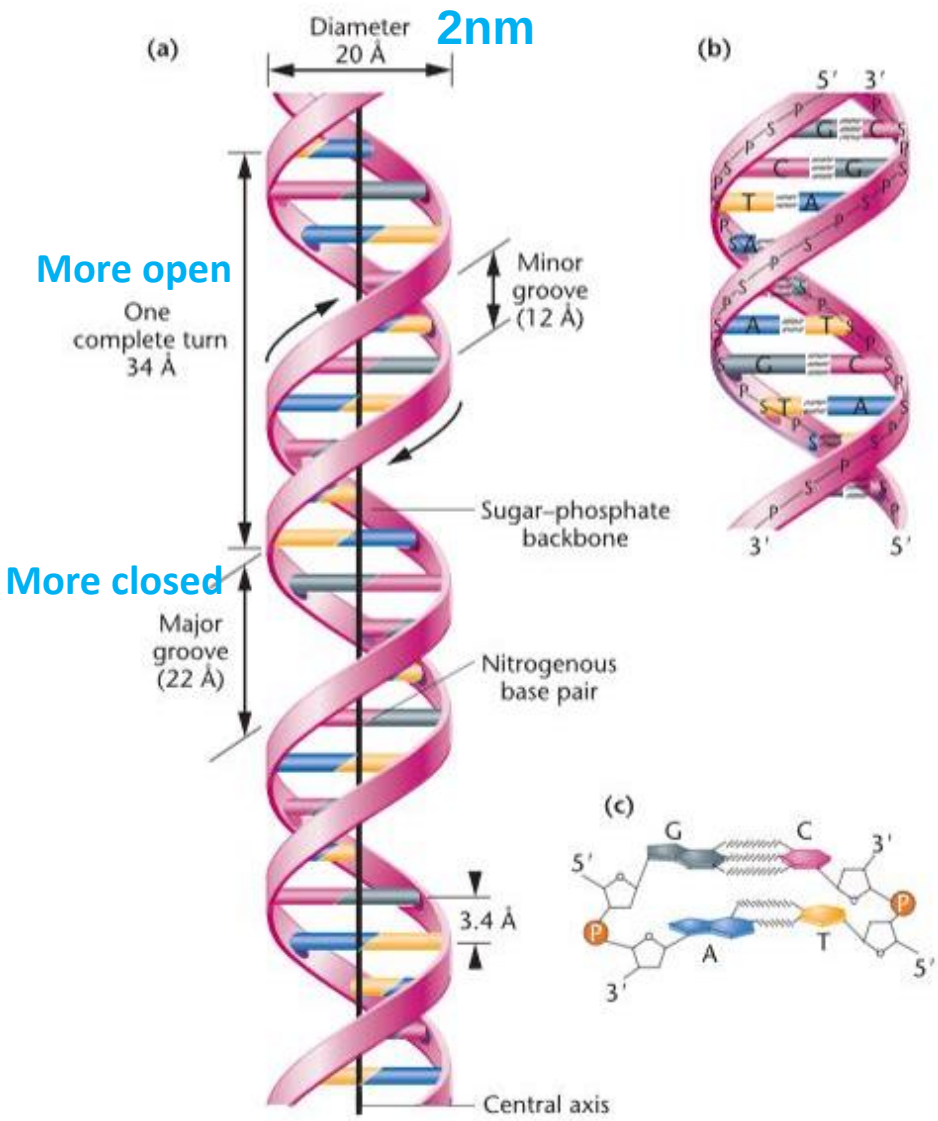
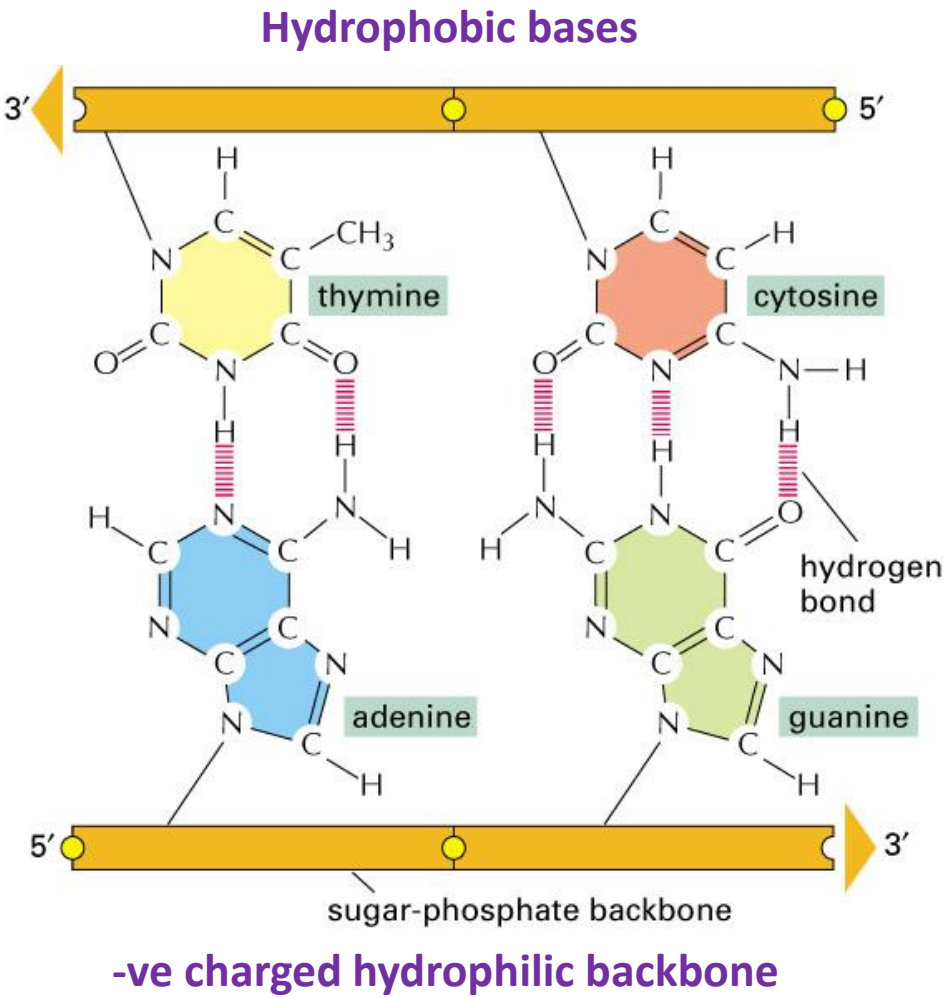


FIGURE 10.12 (a) The DNA double helix as proposed by Watson and Crick. The ribbon-like strands represent the sugar-phosphate backbones, and the horizontal rungs depict the nitrogenous base pairs, of which there are 10 per complete turn. The major and minor grooves are apparent. A solid vertical line shows the central axis. (b) A detailed view depicting the bases, sugars, phosphates, and hydrogen bonds of the helix. (c) A demonstration of the antiparallel arrangement of the chains and the horizontal stacking of the bases.

Stability of the helix

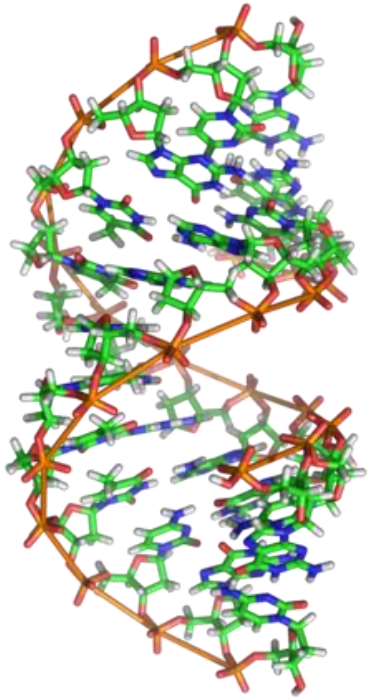
- The stability of the helix is due to:
  - Large number of hydrogen bonds
  - GC rich DNA is more stable than AT-rich DNA because the GC base pair has three hydrogen bonds
  - Hydrophobic bonding (base stacking) between bases- trying to force water out
  - Hydrogen bonds between bases and water molecules



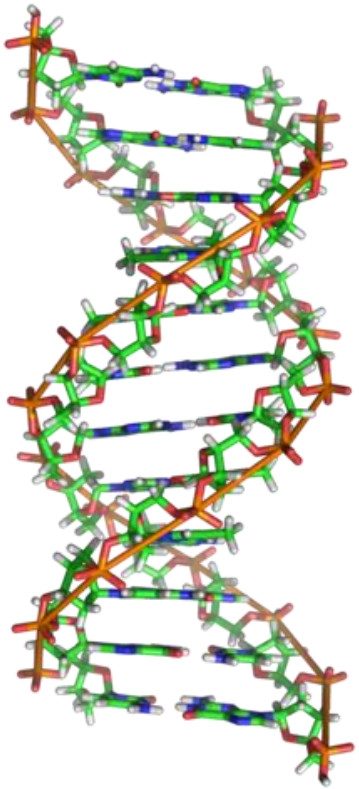
DNA secondary structure

Three biologically active double helical structures

A-DNA

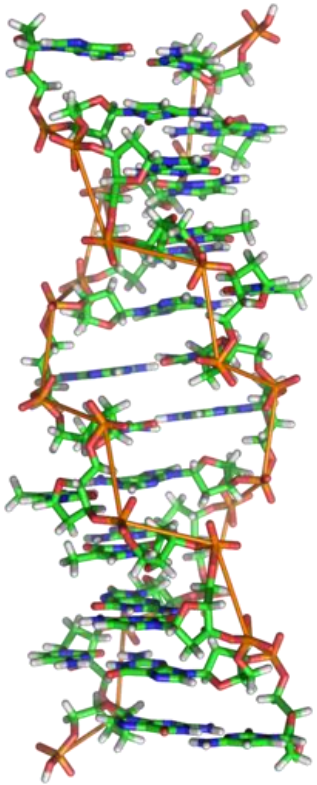


B-DNA



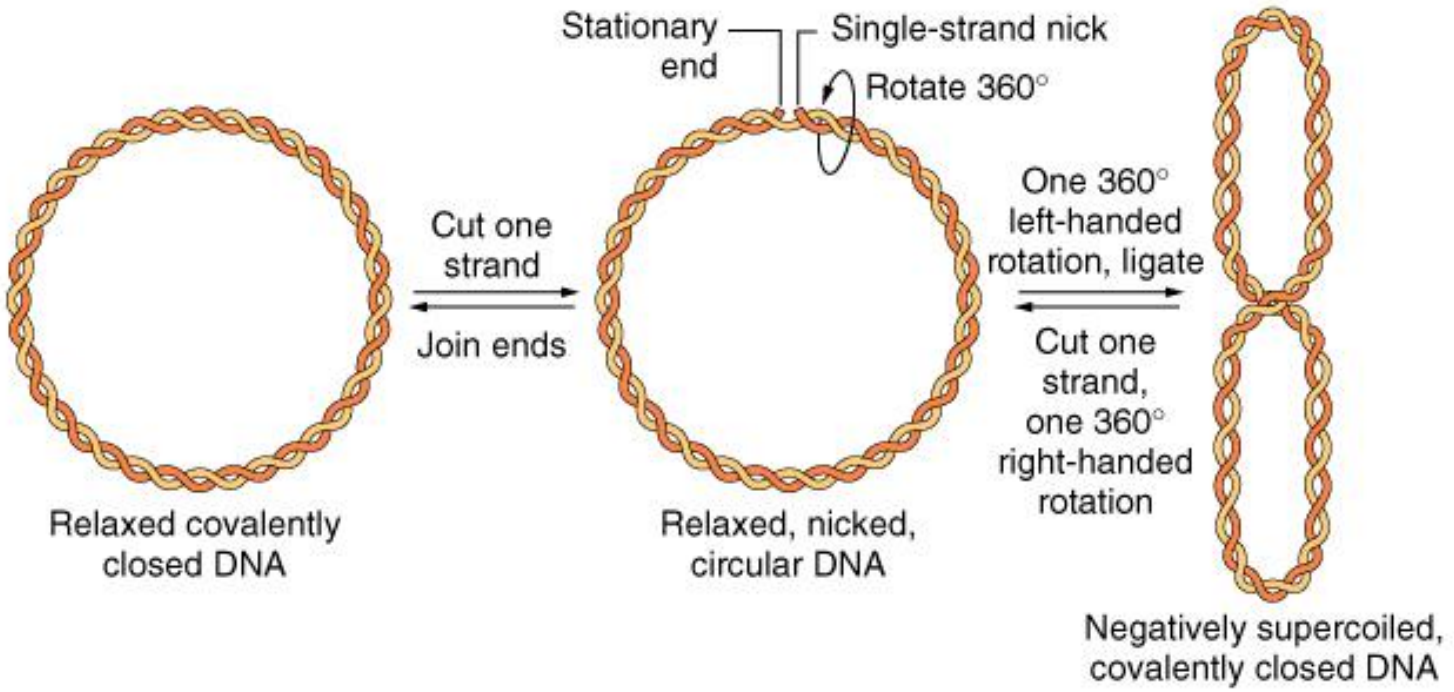
Most common – first described, right handed double helix

Z-DNA

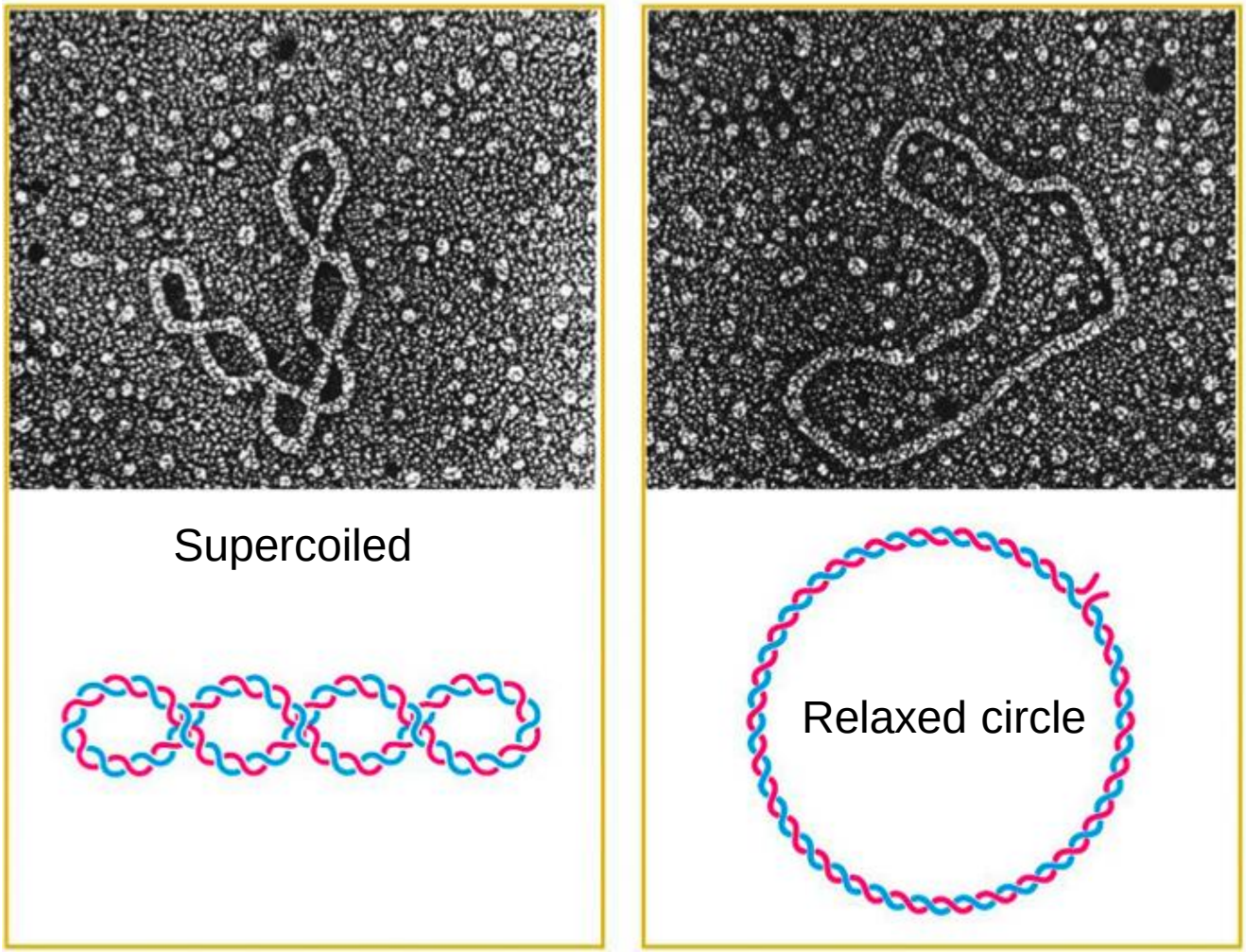


Bacteria: DNA can be negatively supercoiled

- The relaxed form of DNA is twisted into the **"biologically active"** (negatively supercoiled structure)
- This state of supercoiled DNA is maintained within the cell by a series of enzymes called topoisomerases

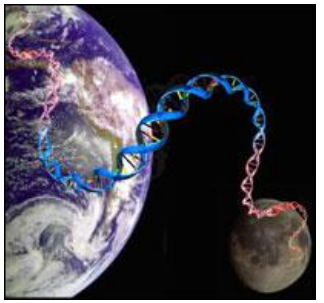


DNA can be negatively supercoiled



DNA is folded to form the chromosome

- If the DNA of **one human cell** was stretched out, it would span 2 metres!
  - How is this folded into a single cell of 5µm in diameter?
  - The **DNA must be packaged**



- At interphase, the DNA is dispersed –**chromatin** so that it can be replicated and expressed but must be condensed into a chromosome during cell division

DNA is folded to form the chromosome

- http://www.youtube.com/watch?v=gbSIBhFwQ4s&feature=relmfu

1.43 min

Chromatin

- The **combination of DNA and proteins** that make up the contents of the nucleus of a cell
- Primary functions:

  1. To **package** DNA into a smaller volume to fit in the cell

  2. To **strengthen** the DNA to allow mitosis and meiosis and prevent DNA damage (strengthen during anaphase separation **– spindle force**)

  3. To **control gene expression** and DNA replication

  - The primary protein components of chromatin are **histones** that compact the DNA

- Orders of chromatin structure from naked DNA to chromatin to fully condensed chromosomes

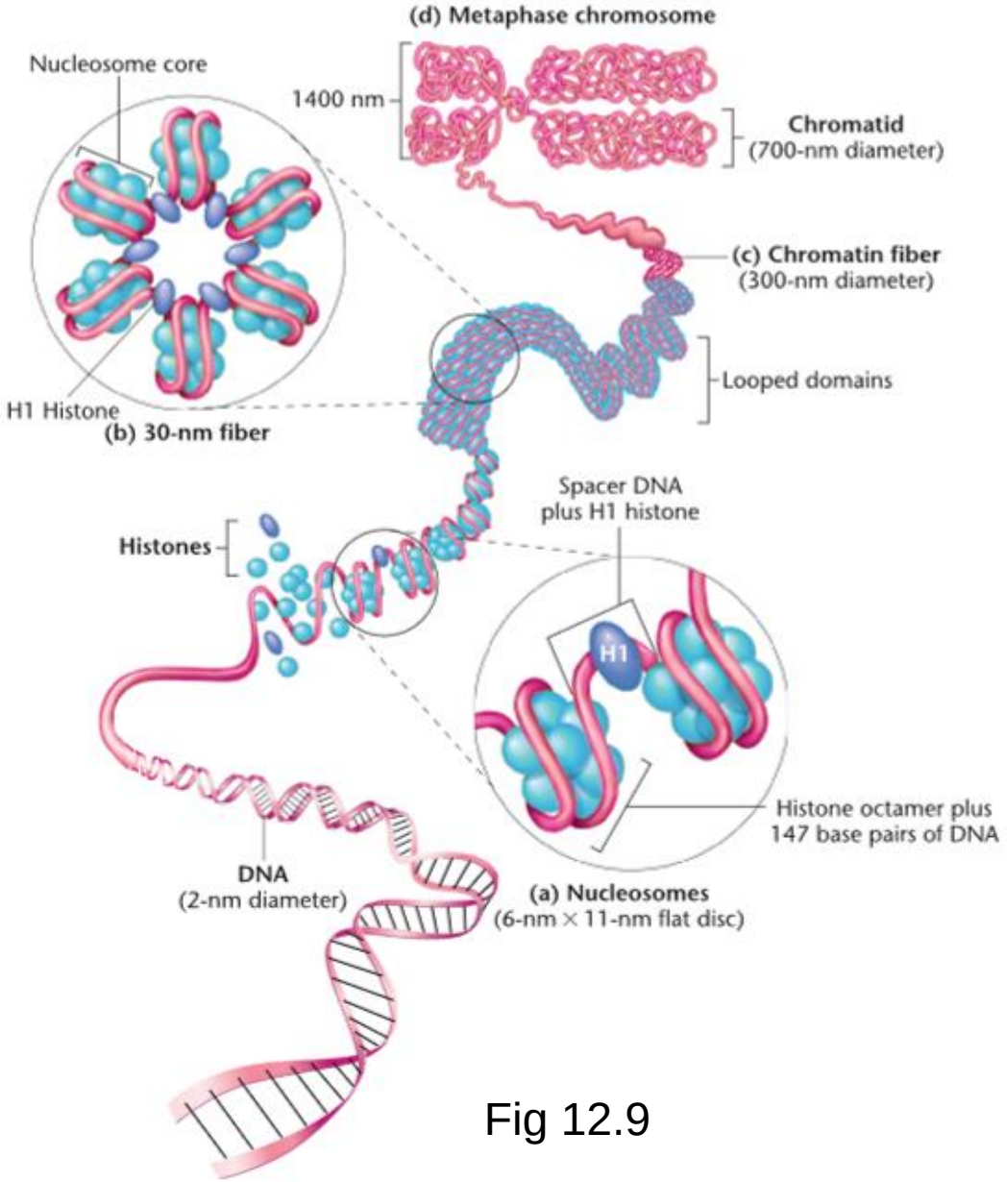
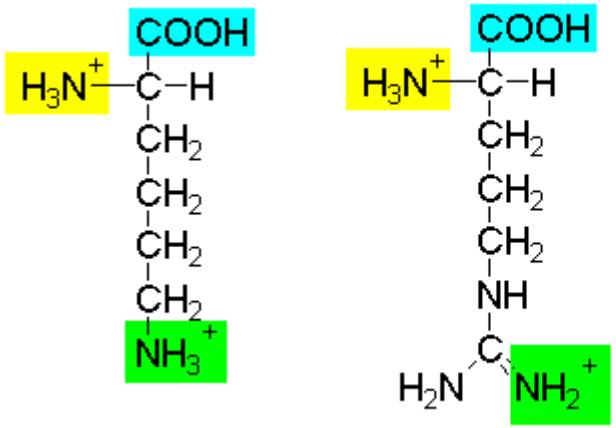


Fig 12.9

Histones

- Abundant molecules
- Histone protein sequence is highly conserved among eukaryotes — conserved function
- Provide the **first level of packaging for DNA**
  - Compact the DNA by a factor of approximately 7
- Rich in Lysine and Arginine (+ve charged, alkaline)



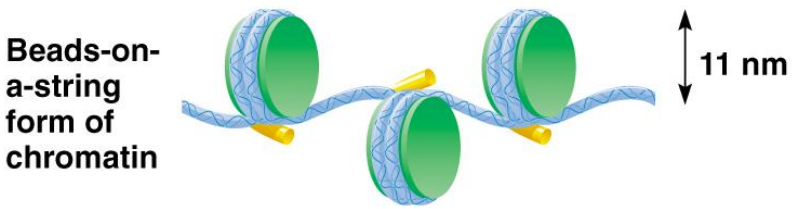
Histone positive charge attracts negatively charged DNA

Histones

- 5 main types
  - H2A
  - H2B
  - H3
  - H4

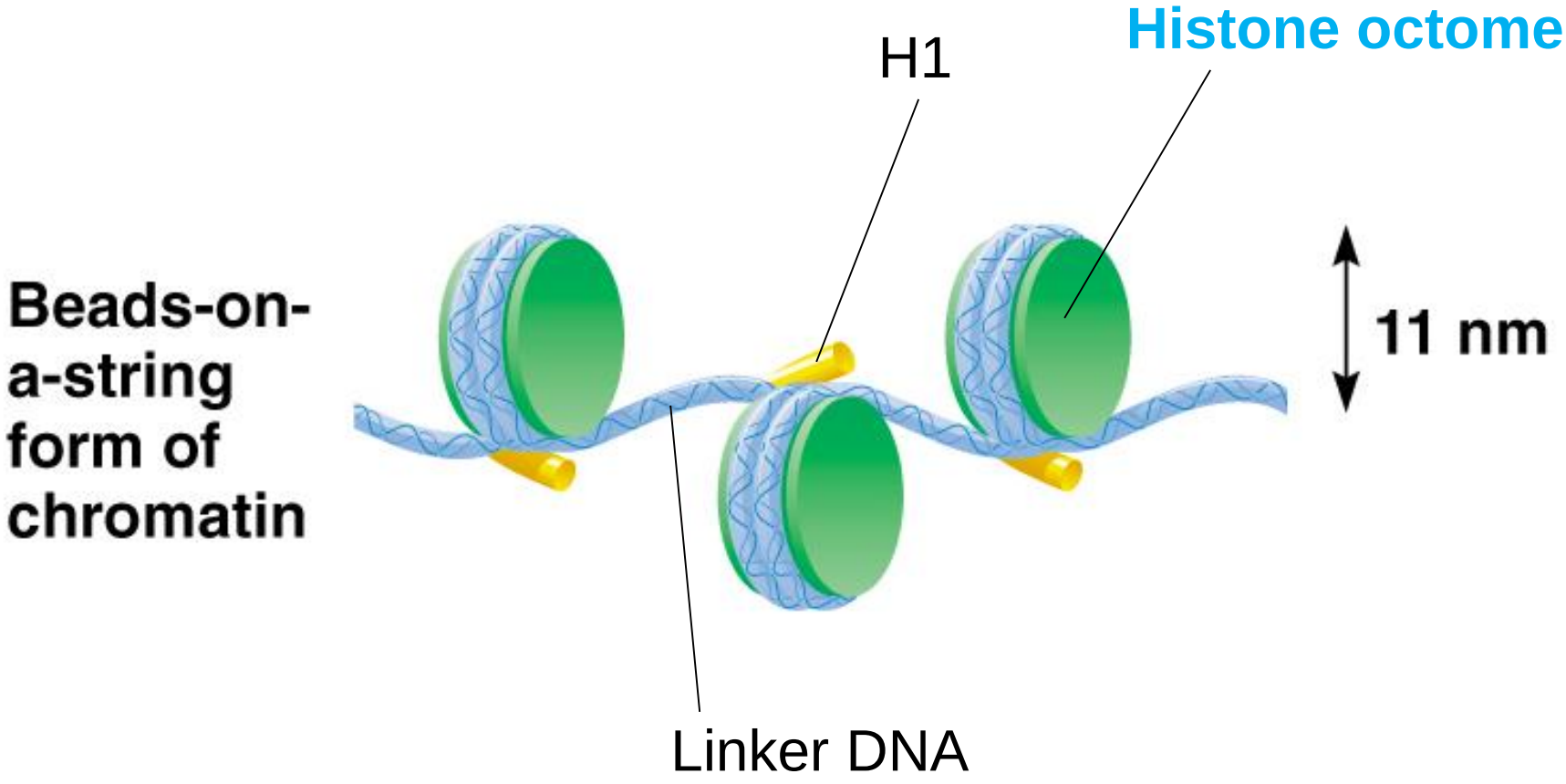
Two copies in each nucleosome ‘histone octomer’; DNA wraps around this structure 1.75 times

- H1? — attached to the nucleosome and involved in further packaging of the DNA (conversion of 10 nm chromatin to 30 nm chromatin)
- The histone structure produces a 10nm "beads-on-a-string" fibre



10nm chromatin

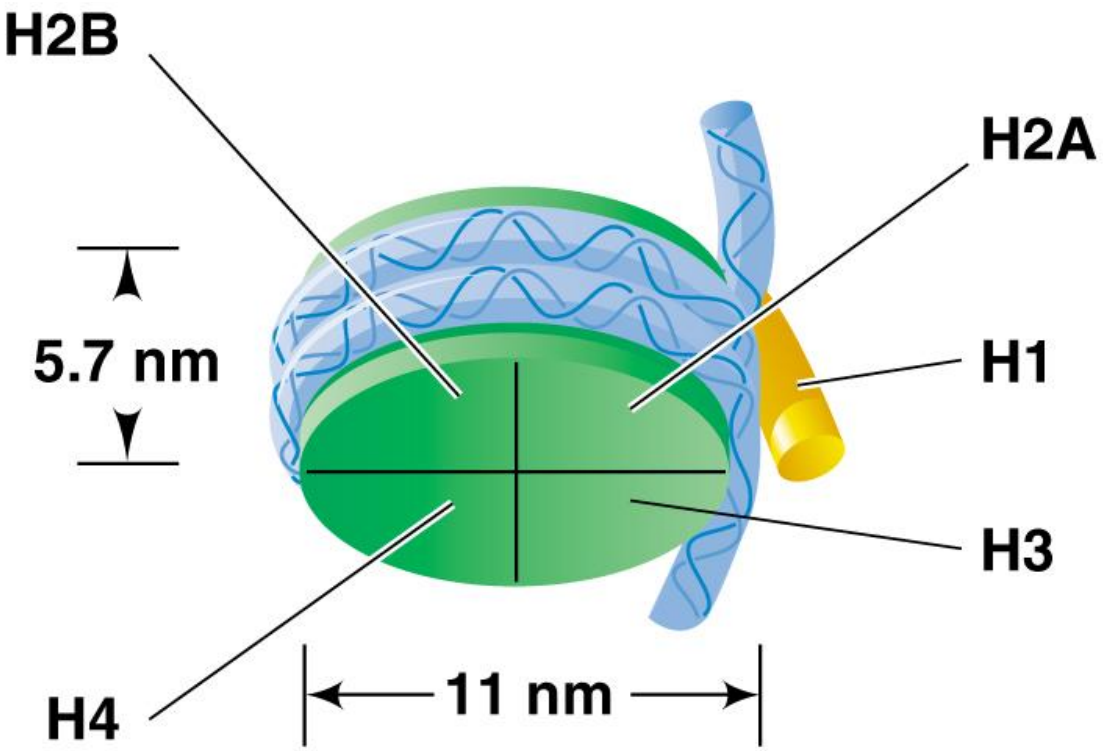
10 nm chromatin is produced in the **first level** of packaging



Euchromatin: regions of accessibility to genes

Nucleosome

- DNA wound in sequence around **eight histone protein** cores



DNA wraps around this structure 1.75 times

Further compaction

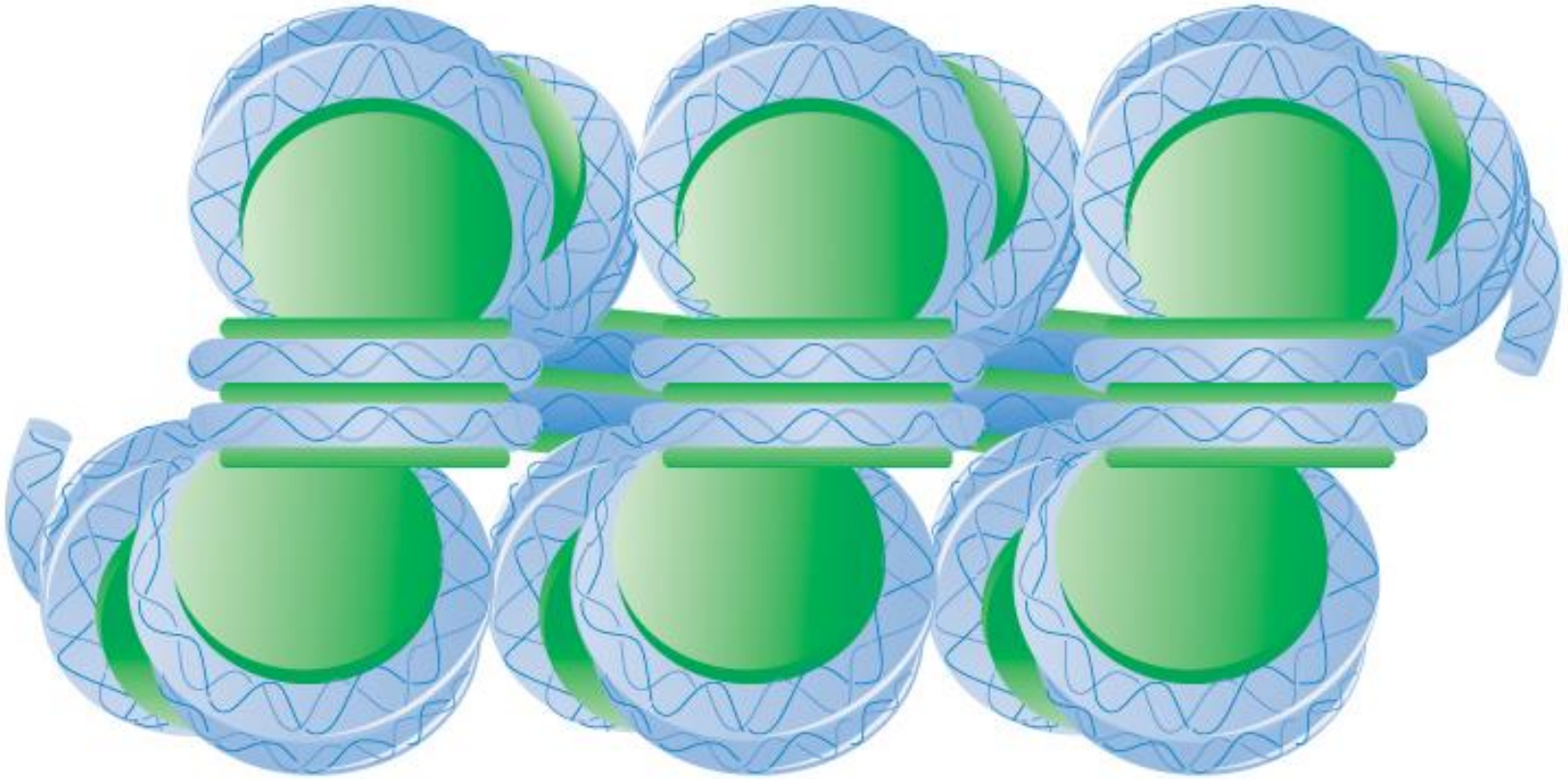
- DNA is further packaged when the DNA nucleosomes associate with one another to produce 30nm chromatin

- Mechanism of packaging is not understood, but H1 plays a role (if H1 is absent, then chromatin cannot be converted from 10 to 30 nm)

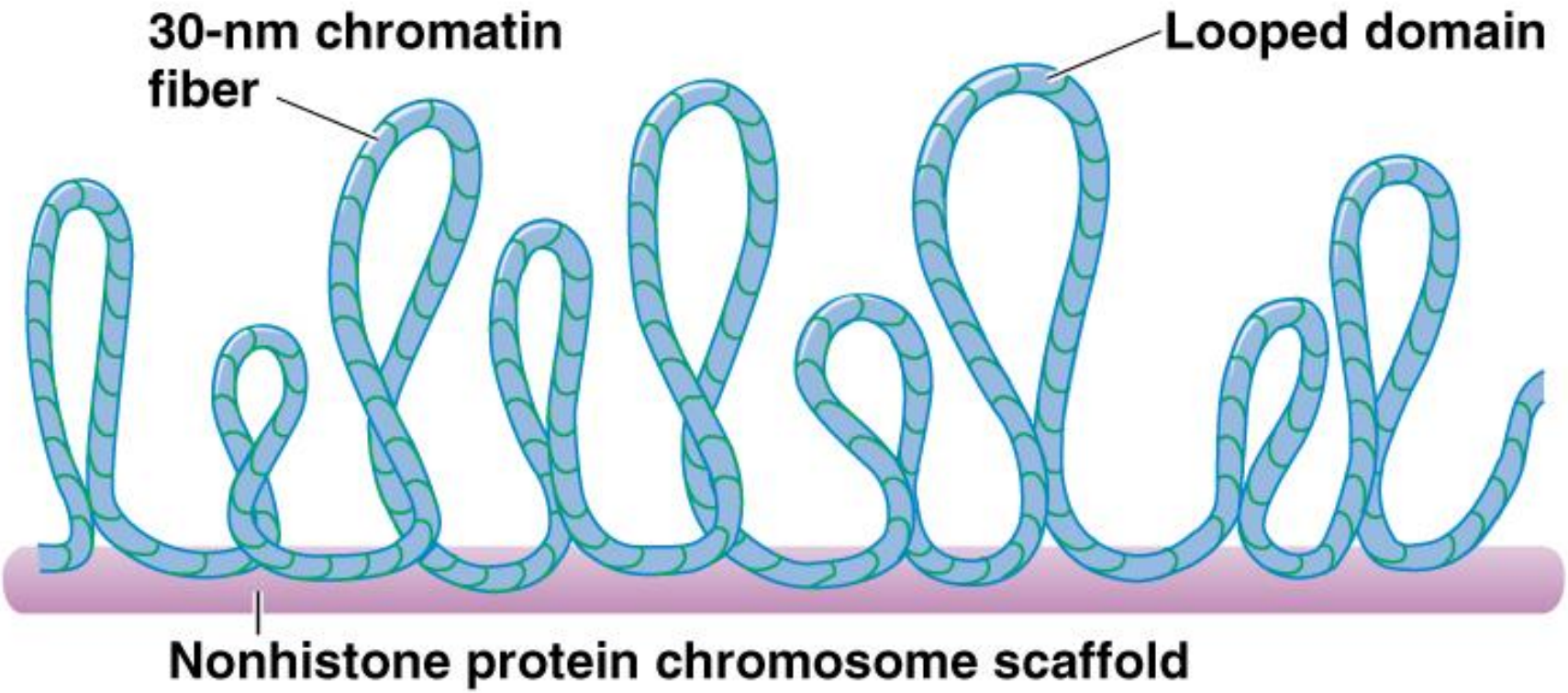
- DNA is condensed to 1/6th its unfolded size, **30nm heterochromatin**

30nm chromatin

Heterochromatin: Genes are inaccessible



Looped domains



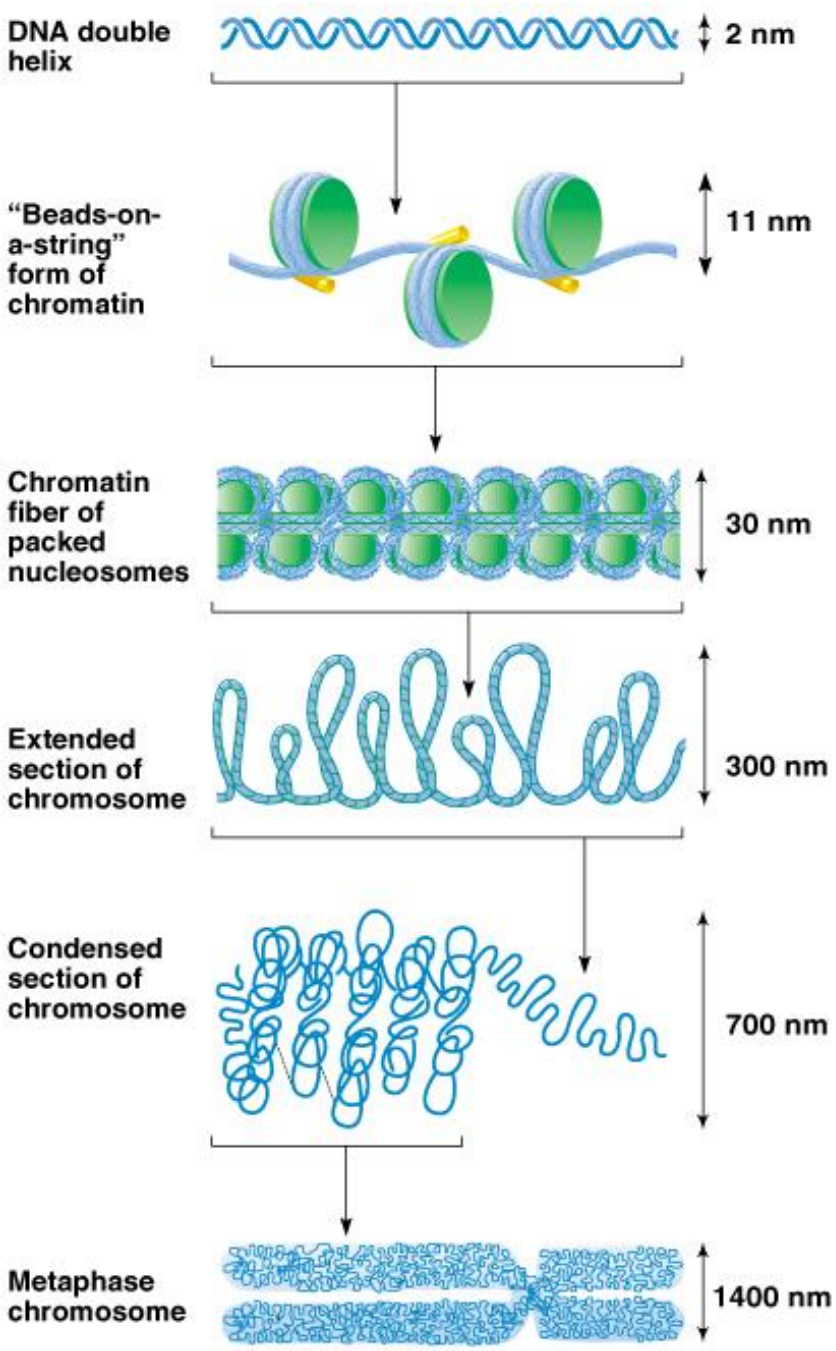
Loops are arranged so that the DNA condensation can be independently controlled for gene expression. Loops (DNA sequence) bind to a nuclear martix, known as MAR (matrix attachment regions)

First stage of packaging: euchromatin

Second stage of packaging: heterochromatin

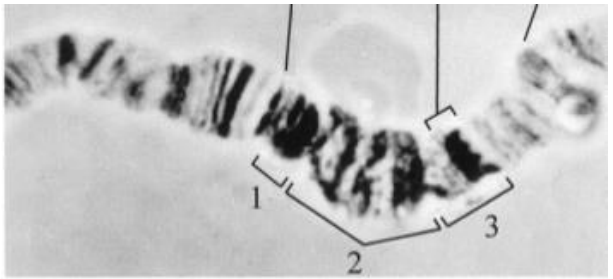
Further packaging

Completely packaged Metaphase chromosome



DNA compaction

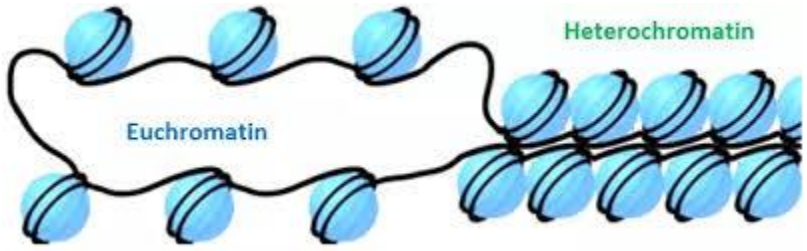
- Level of DNA compaction changes throughout the cell cycle;
  - Most compact during Mitosis and least compact during Synthesis
- 2 types of chromatin; related to the level of gene expression
  - Euchromatin—defined originally as areas that stained lightly
  - Heterochromatin—defined originally as areas that stained darkly



Euchromatin / Heterochromatin

- Euchromatin — chromosomes or regions therein that exhibit normal patterns of condensation and relaxation during the cell cycle
  - Most areas of chromosomes in active cells
  - Usually areas where gene expression is occurring

- Heterochromatin — chromosomes or regions therein that are condensed throughout the cell cycle
  - Provided first clue that parts of eukaryotic chromosomes do not always encode proteins.

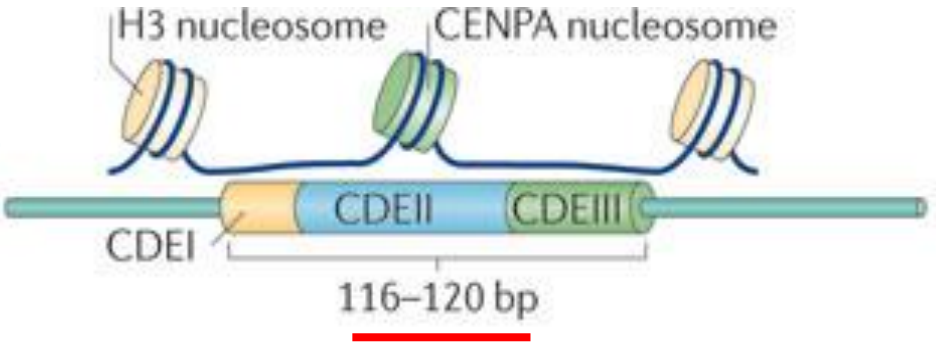


Heterochromatin: Centromere

- Part of a chromosome that **links sister chromatids**
- Act as the site of assembly of **the kinetochore**
- **Two types:**
- Point centromeres
  - Very small regions of DNA (~125 bp in length)
  - Capable of genetically **conferring centromeric function** on any DNA segment into which they are transferred to
  - Seen in the budding yeast, *Saccharomyces cerevisiae*

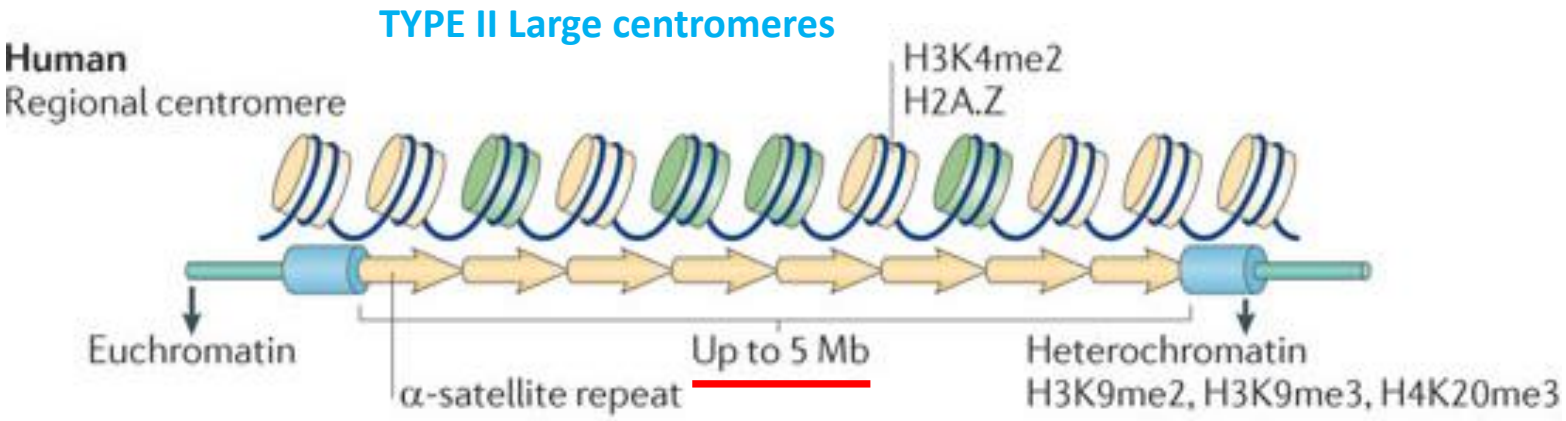
Budding yeast
Point centromere

TYPE I Point centromeres



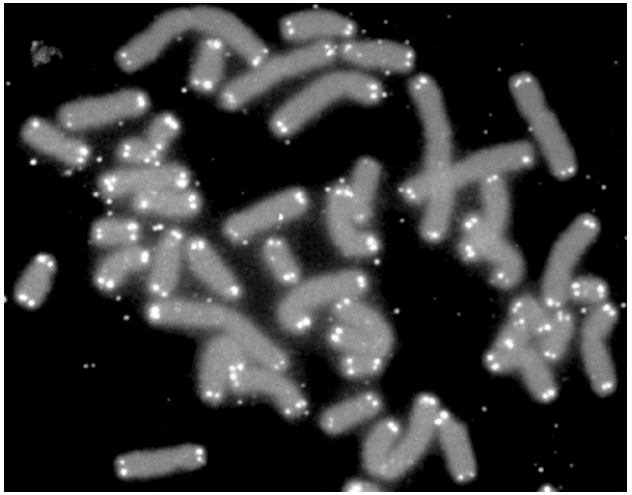
Centromere

- Human and mammalian regional centromeres contain α satellite DNA
  - **171bp** sequence arranged in **tandem repeats**
  - Binds to centromere-associated proteins
- Large centromeres **bind 30-40 microtubules**
  - Point centromere of *S. cerevisiae* **binds one**



Telomere

- The sequences at the **ends** of eukaryotic chromosomes
  - Play critical roles in chromosome replication and maintenance
  - Telomeres keep chromosomes protected and prevent them from fusing into rings or binding with other DNA



Telomere

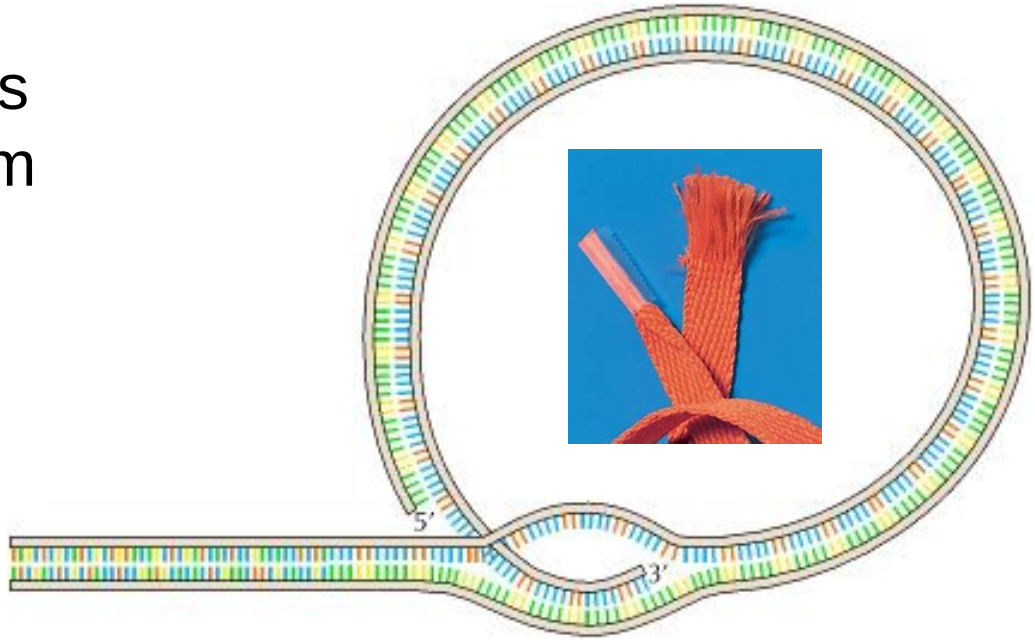
- The telomere DNA sequences of a variety of eukaryotes are largely **conserved**, consisting of repeats of a simple-sequence DNA containing clusters of G residues on one strand

  - E.g. the sequence of telomere repeats in humans and other mammals is AGGGTT, in *Tetrahymena* (a protozoan) it is GGGGTT, in *Arabidopsis* (a plant) it is AGGGTTT

Telomere

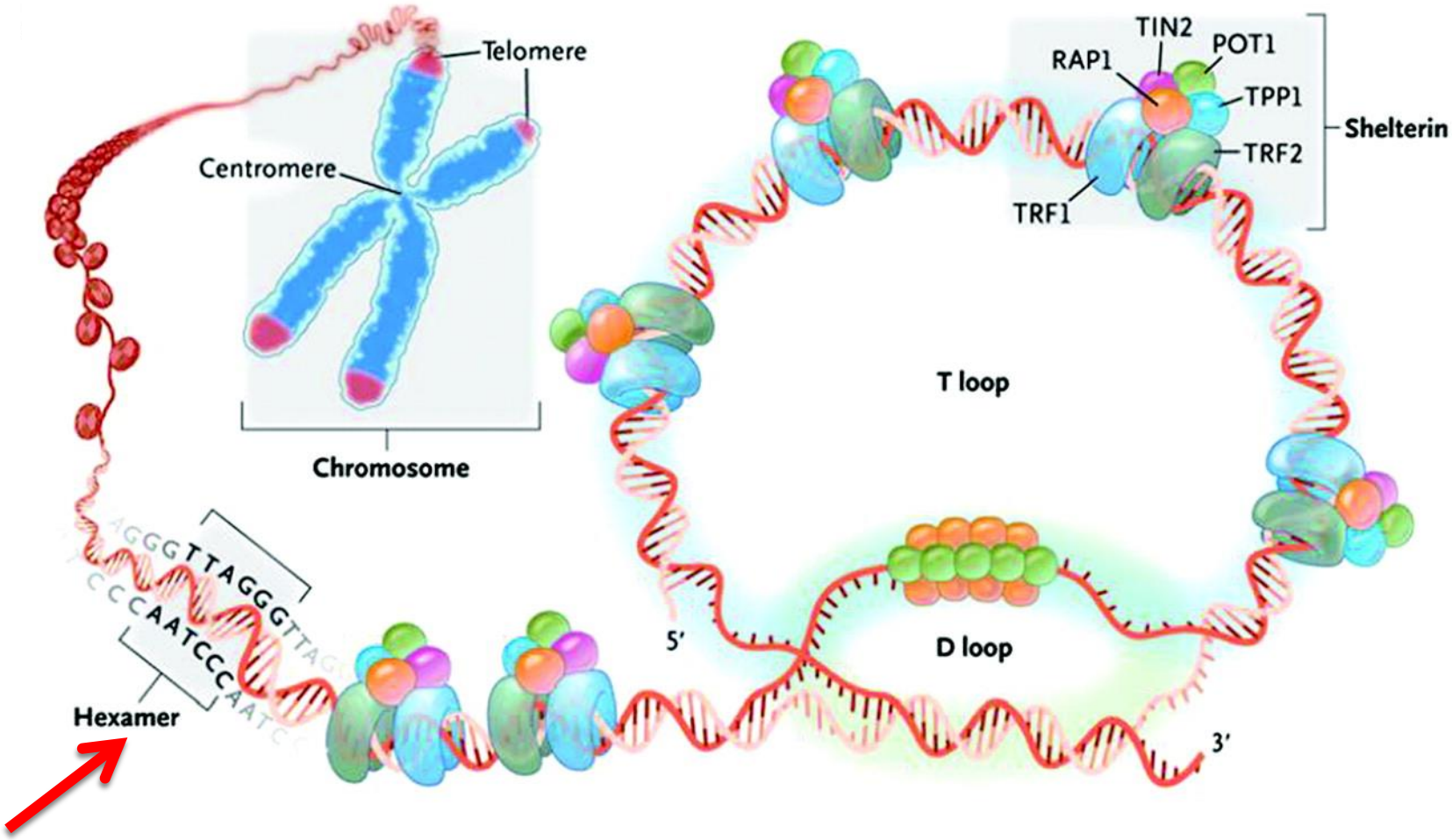
- These sequences are repeated **hundreds or thousands of times,**
  - Spanning up to several kilobases,
  - Terminate with an overhang of single-stranded DNA

Telomere DNA loops back on itself to form a circular structure that protects the ends of chromosomes- chromosomes are sealed at the ends

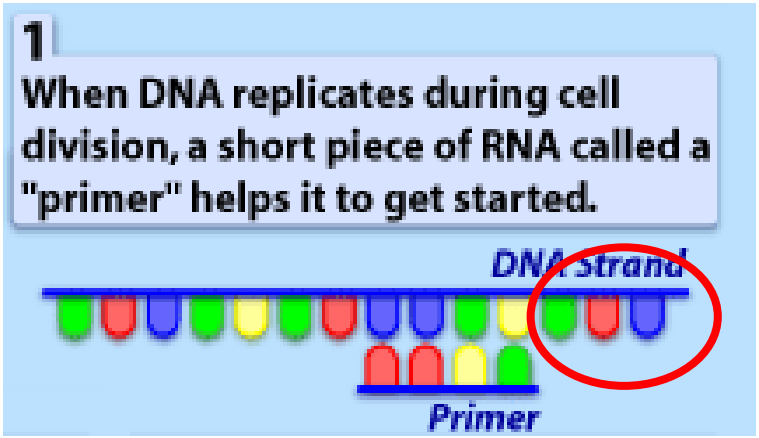


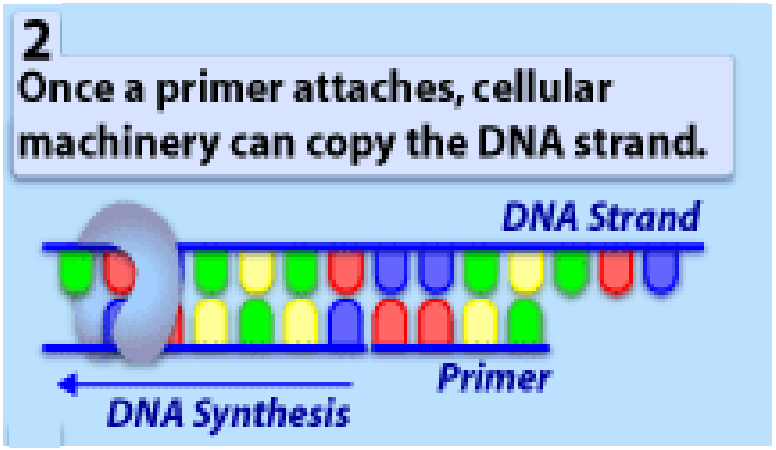
Telomere

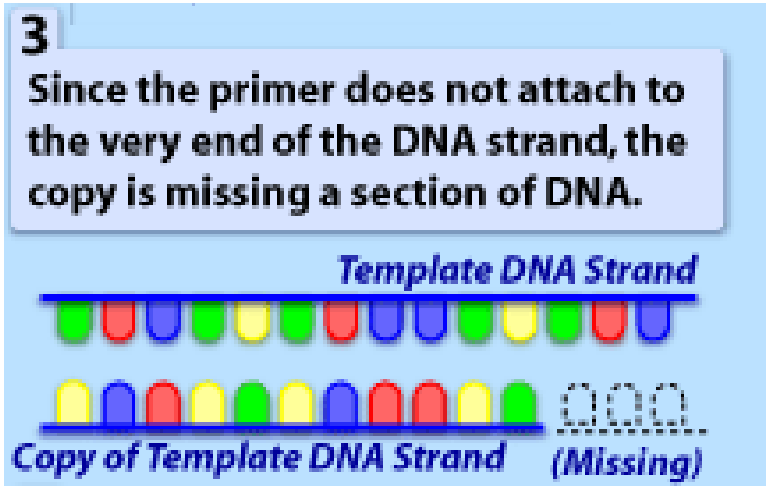
Shelterins protect and control telomere copy number

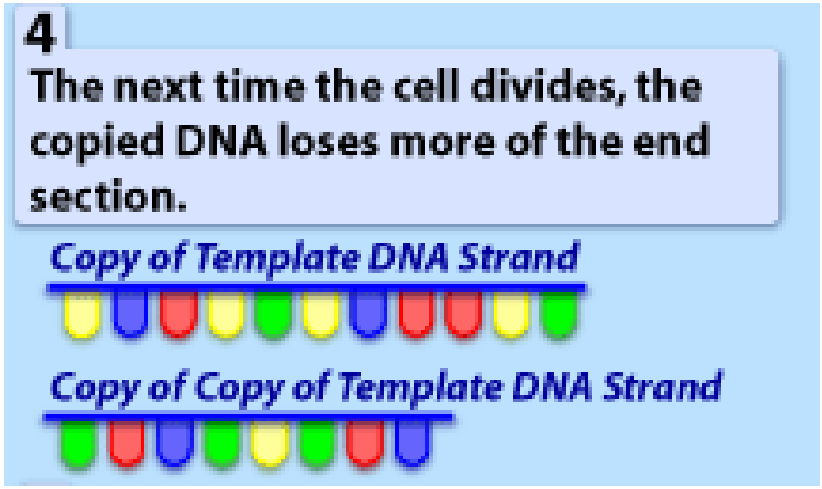


Why are telomeres so important?
Telomere shortening





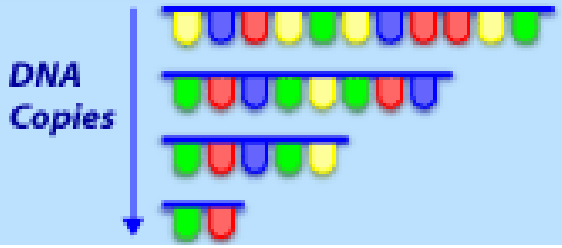




Telomere shortening

5

As cell division continues, the end section of each new DNA strand gets shorter and shorter. In humans,about 50 - 250 base pairs are lost per cell division.



7

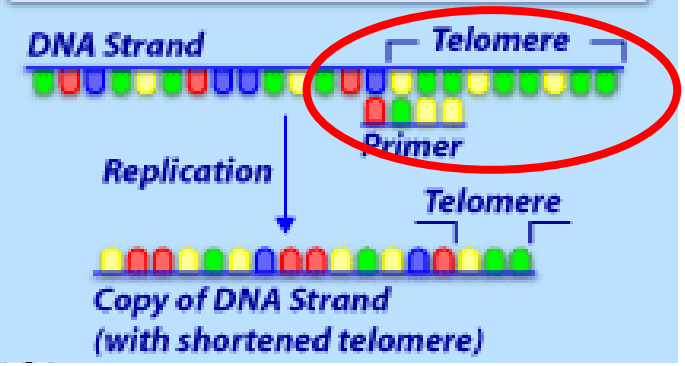
In germline cells (egg and sperm), an enzyme called telomerase is responsible for adding more repeat sequences to the end of the DNA, thus making them "immortal".

How do our cells have any DNA left?

TELOMERES!

6

Telomeres are repeated sequences on the ends of DNA strands. They help protect the DNA strand from getting shorter during cell division.

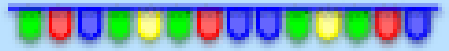


Telomere shortening

8

In somatic cells, the telomerase enzyme functions at much lower levels, making these cells "mortal".

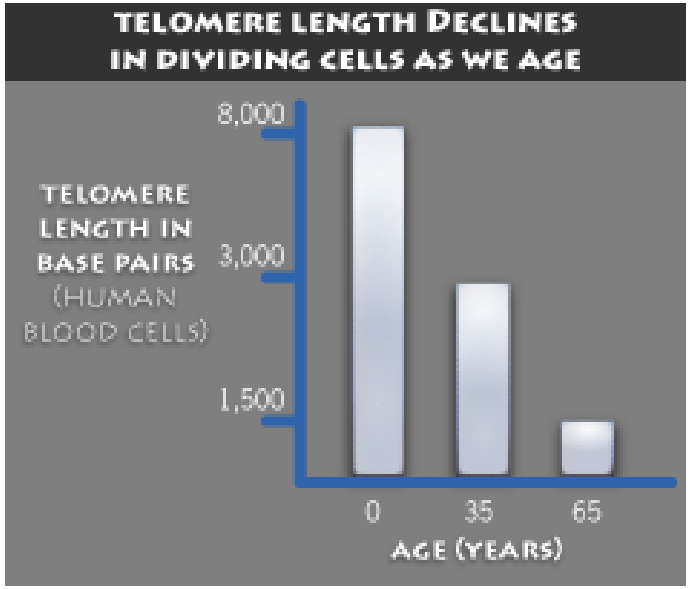
DNA from old somatic cell (no telomere left)



9

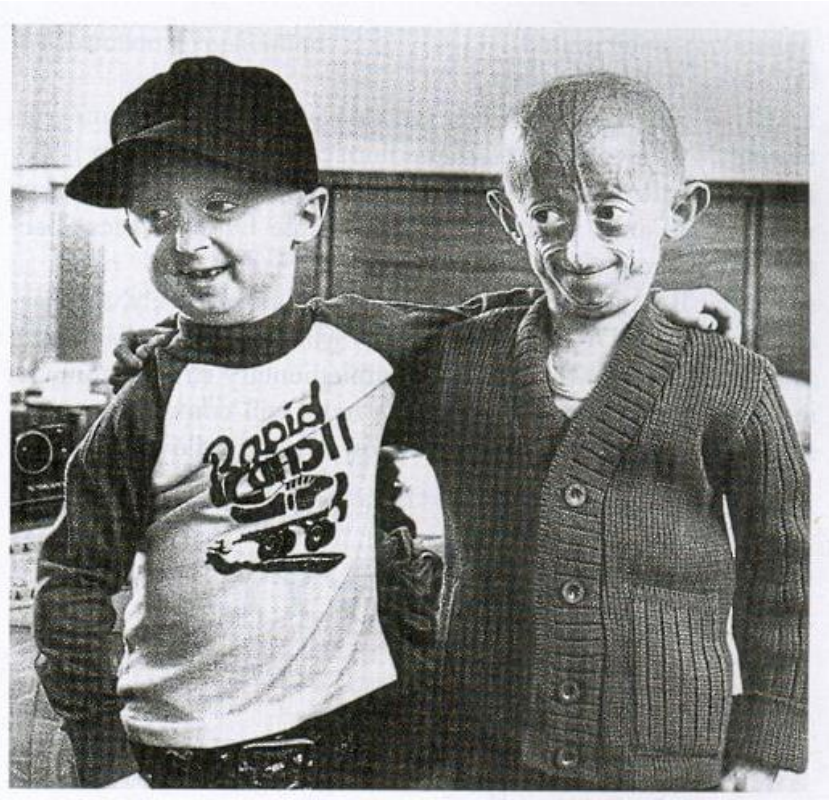
When the telomeres in a somatic cell shorten to a critical level, that cell no longer divides. This phenomenon contributes to some of the changes we see in aging.

- Maintenance of telomeres appears to be an important factor in determining the lifespan and reproductive capacity of cells



Telomere and ageing

- Accelerated ageing diseases in humans- progerias
- Patients with Hutchison-Gilford syndrome show symptoms of ageing in childhood and die when teenagers
- Somatic cells from patients with progerias have shorter telomeres



Telomere and cancer

- **Why don’t we have telomerase in our somatic cells?**
- **Because the cells would be able to divide continuously…**
- If telomeres always shorten as a cell divides, and the cell dies if the telomere gets too short, how can a cancer cell live forever and continually divide?
  - Cancer cells **up-regulate telomerase**, which can prevent telomeres from getting shorter and **even elongate them**
  - Telomerase is activated in approximately 90% of tumours

Telomere and cancer