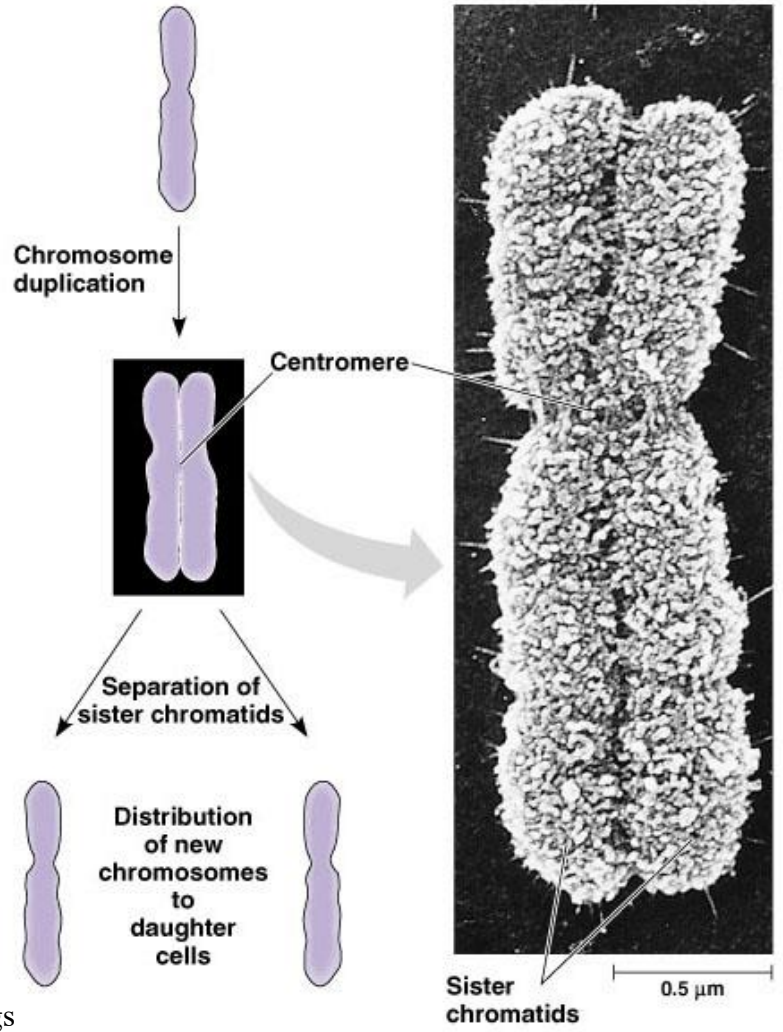
Mitosis

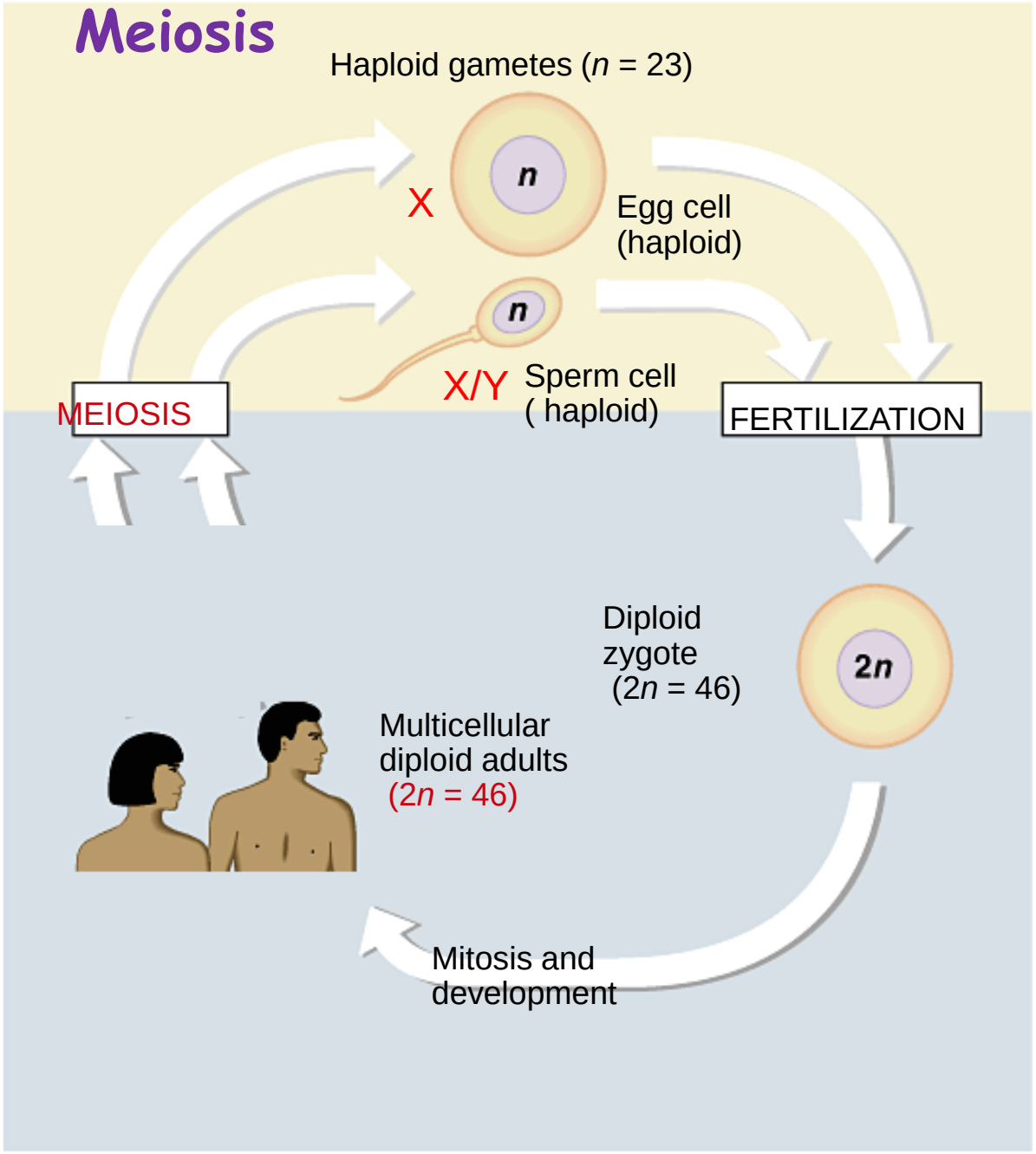
- The process of the formation of the two daughter nuclei which has exact number of chromosomes, but single sister chromatid.
- These processes take one cell and produce two cells that are the genetic equivalent of the parent.

- The fertilized egg or zygote underwent trillions of cycles of mitosis and cytokinesis to produce a fully developed multicellular human.
- These processes continue every day to replace dead and damaged cell.
- Essentially, these processes produce clones - cells with the same genetic information.



Life cycle: Meiosis

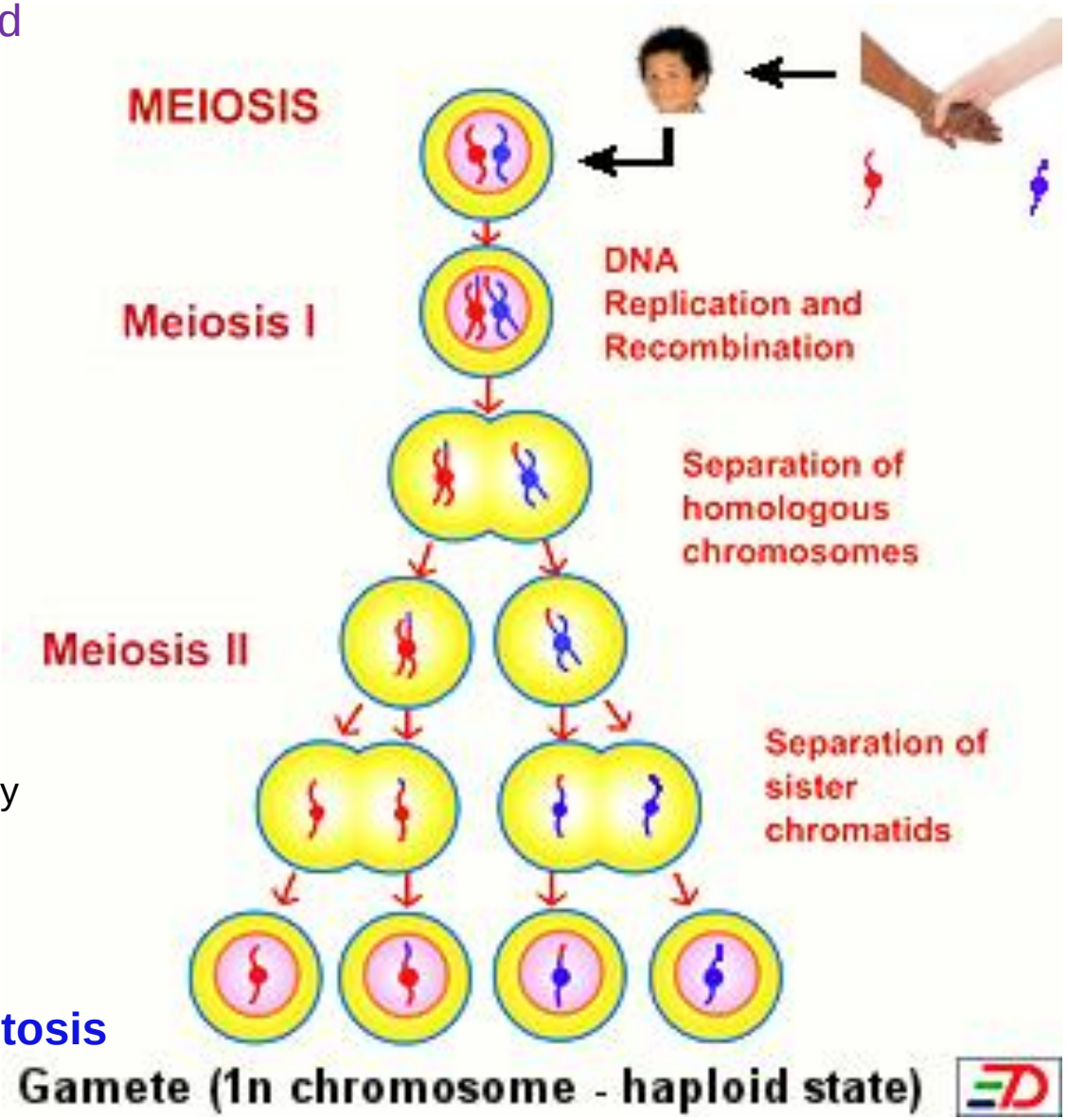
- The human life cycle
- Meiosis is a special form of cell division that produces gametes
- In contrast, gametes (eggs or sperm) are produced only in gonads (ovaries or testes).
- Each gamete has a single set of (haploid) chromosomes
- End of meiosis daughter cells (sperm or egg) has 22 Autosomes and a single sex chromosome (allosome)
- Fertilization fuses two gametes together and doubles the number of chromosomes to 46 again.



An overview of meiosis

Have you wondered how two siblings have same parents and still look so different?

- Meiosis involves 2 cell divisions
- Meiosis produces 4 cells from 1 parental cell
- Each of the 4 daughter cells has **23 individual chromosomes rather than 23 pairs of chromosomes**, and each chromosome has one sister chromatid.
- Meiosis reduces the chromosome number from diploid to haploid
- Exchange of DNA (genetic materials) between homologous chromosomes: Leads to variation
- Meiosis, like mitosis, is preceded by chromosome duplication
  - However, in meiosis the cell divides twice to form four daughter cells



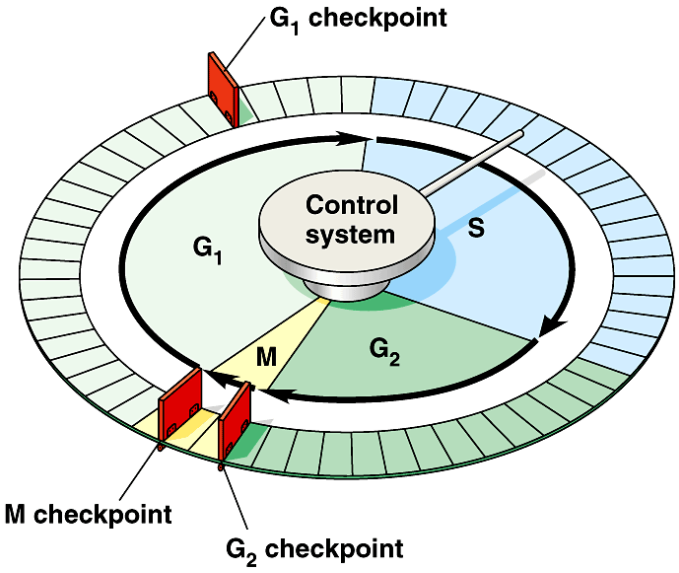
Meiosis ~ Mitosis

DNA content and chromosome numbers?

Cell cycle

The cell cycle or **cell-division cycle** is the series of events that take place in a cell leading to its **division and duplication of its DNA (DNA replication)** to produce two daughter cells.

•**G1 phase.** Metabolic changes prepare the cell for division. At a certain point decides whether the cell is committed to division and moves into the S phase.
•**S phase**. DNA synthesis replicates the genetic material. Each chromosome now consists of two sister chromatids.
•**G2 phase.** Metabolic changes assemble the cytoplasmic materials necessary for mitosis and cytokinesis.
•**M phase.** A nuclear division (mitosis) followed by a cell division (cytokinesis).



What's the outcome of the S phase cell fused to G1 phase cell?
What's the outcome of the G1 phase cell fused to M phase cell?

What controls the cell cycle?

Are there 'molecular signals' in the cell that regulate the cell cycle?

What controls the cell cycle?

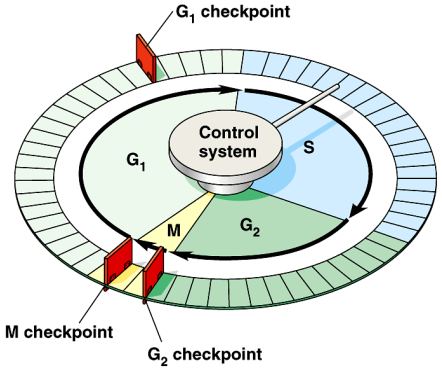
Hypothesis: Each event in the cell cycle merely leads to the next as in a simple metabolic pathway.

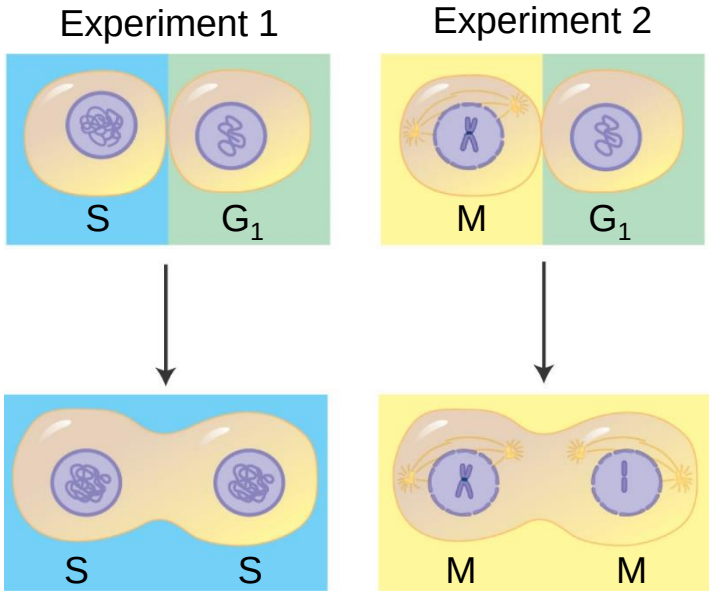
Alternative hypothesis: Cycle is driven by specific signaling molecules .

EXPERIMENTS

In each experiment, cultured mammalian cells at two different phases of the cell cycle were induced to fuse.

RESULTS





When a cell in the S phase was fused with a cell in G_1, the G_1 cell immediately entered the S phase—DNA was synthesized.

When a cell in the M phase was fused with a cell in G_1, the G_1 cell immediately began mitosis—a spindle formed and chromatin condensed, even though the chromosome had not been duplicated

CONCLUSION

The results of fusing cells at two different phases of the cell cycle suggest that molecules present in the cytoplasm of cells in the S or M phase control the progression of phases.

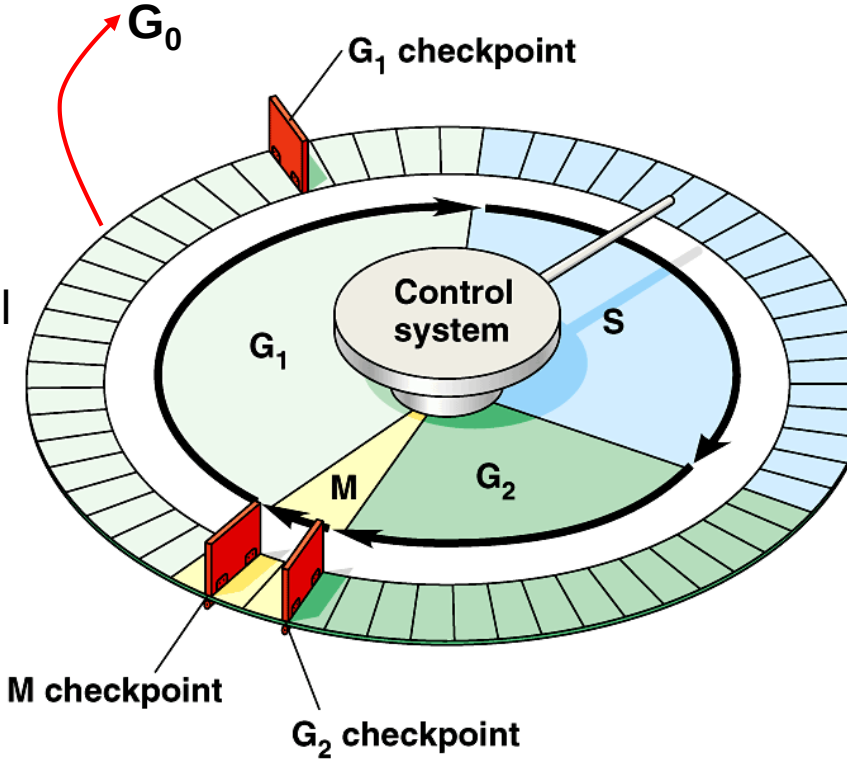
- The distinct and sequential events of the cell cycle are directed by a distinct **cell cycle control system**.
  - Cyclically operating set of molecules trigger and coordinate key events in the cell cycle.
  - The control cycle has a built-in clock, but it is also regulated by external adjustments and internal controls.
  - Animal cells generally have

  built-in stop signals that halt the cell cycle at checkpoints until overridden by

  go ahead signals.

- Most cells in our body are in **G0 phase**.

- A **checkpoint** in the cell cycle is a critical control point where stop and go signals (like traffic signals) regulate the cycle.

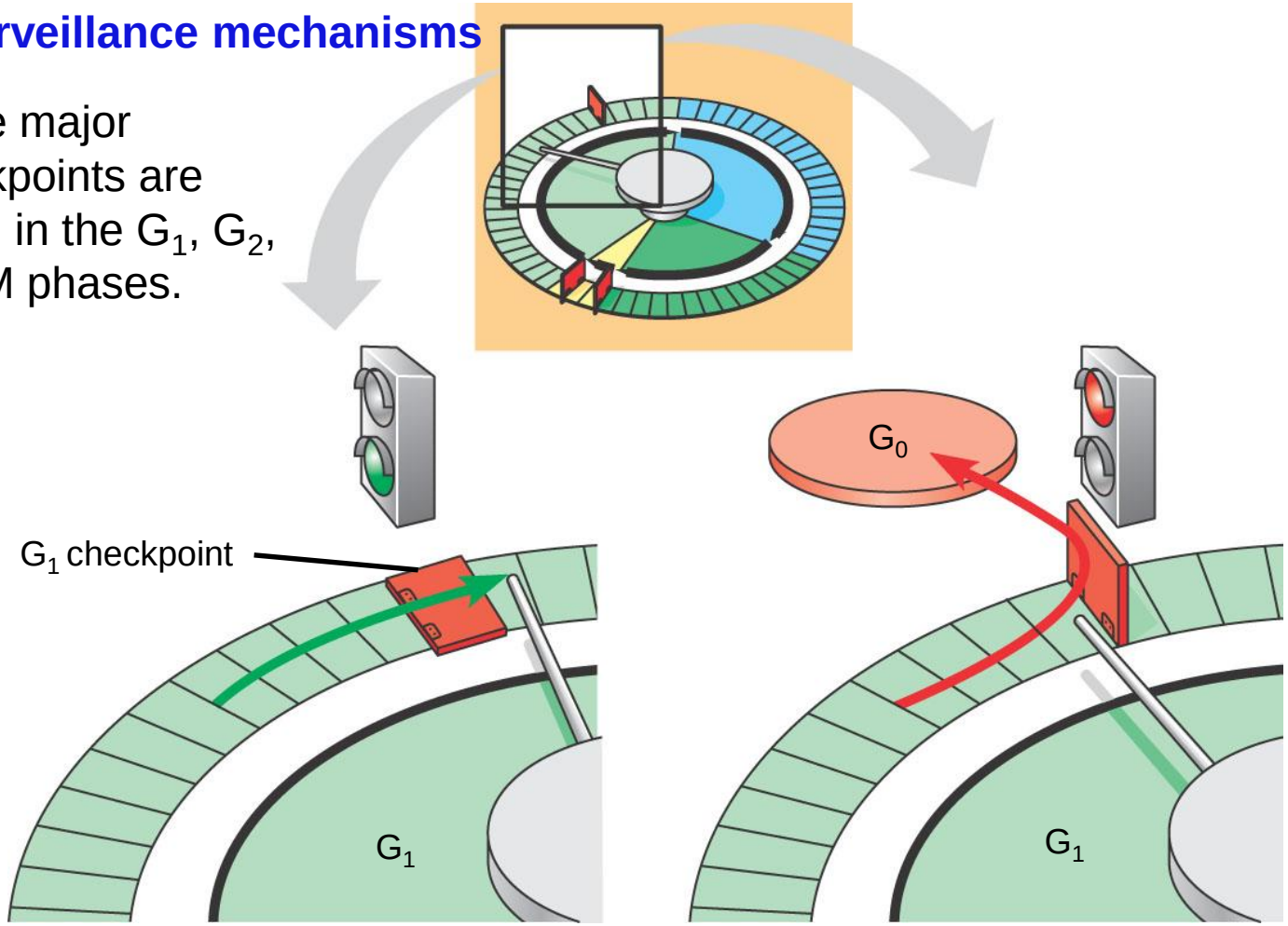


To understand how cell cycle checkpoints work, we 1st need to **see what kinds of molecules make up the cell cycle control** system and how a cell progress **through the cycle**. Then we will consider the internal and external checkpoint signals that can make the clock pause or continue.

The G_1 checkpoint: Stop and Go signals

Cell Surveillance mechanisms

- Three major checkpoints are found in the G_1, G_2, and M phases.



(a) If a cell receives a go-ahead signal at the G_1 checkpoint, the cell continues on in the cell cycle.

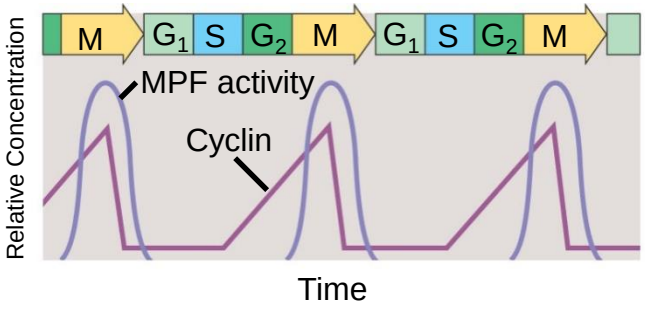
(b) If a cell does not receive a go-ahead signal at the G_1checkpoint, the cell exits the cell cycle and goes into G_0, a nondividing state.

Muscle cells, Nerve cells

Cancer?

Molecular control of the cell cycle at the G_2 checkpoint

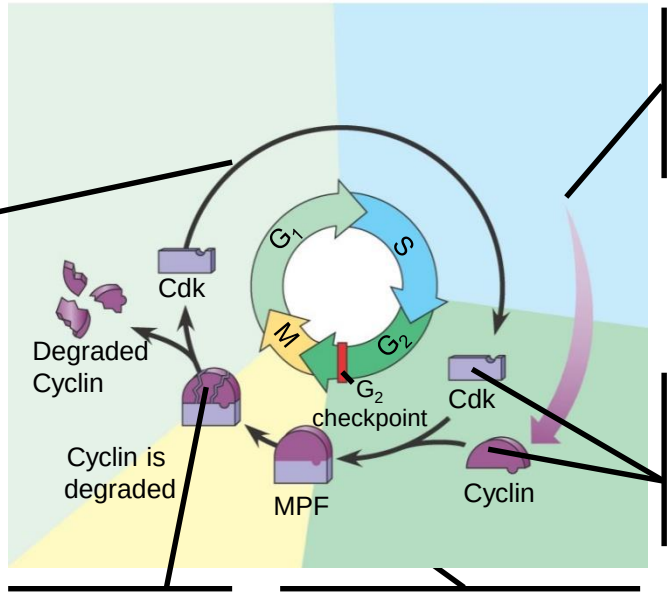
(a) Fluctuation of MPF activity and cyclin concentration during the cell cycle



MPF: M-phase promoting factor (Cyclin + Cdk)

Cdk: cyclin dependent kinase

(b) Molecular mechanisms that help regulate the cell cycle



1 Synthesis of cyclin begins in late S phase and continues through G_2. Because cyclin is protected from degradation during this stage, it accumulates.

2 Accumulated cyclin molecules combine with recycled Cdk molecules, producing enough molecules of MPF to pass the G_2 checkpoint and initiate the events of mitosis.

3 MPF promotes mitosis by phosphorylating various proteins. MPF's activity peaks during metaphase.

4 During anaphase, the cyclin component of MPF is degraded, terminating the M phase. The cell enters the G_1 phase.

5 During G_1, conditions in the cell favor degradation of cyclin, and the Cdk component of MPF is recycled.

Similar mechanisms at G1 check point

Nobel Prize in Physiology or Medicine: **Sir Paul Maxime Nurse,** Leland H. Hartwell and R. Timothy Hunt: discoveries of protein molecules that control the division (duplication) of cells in the cell cycle.

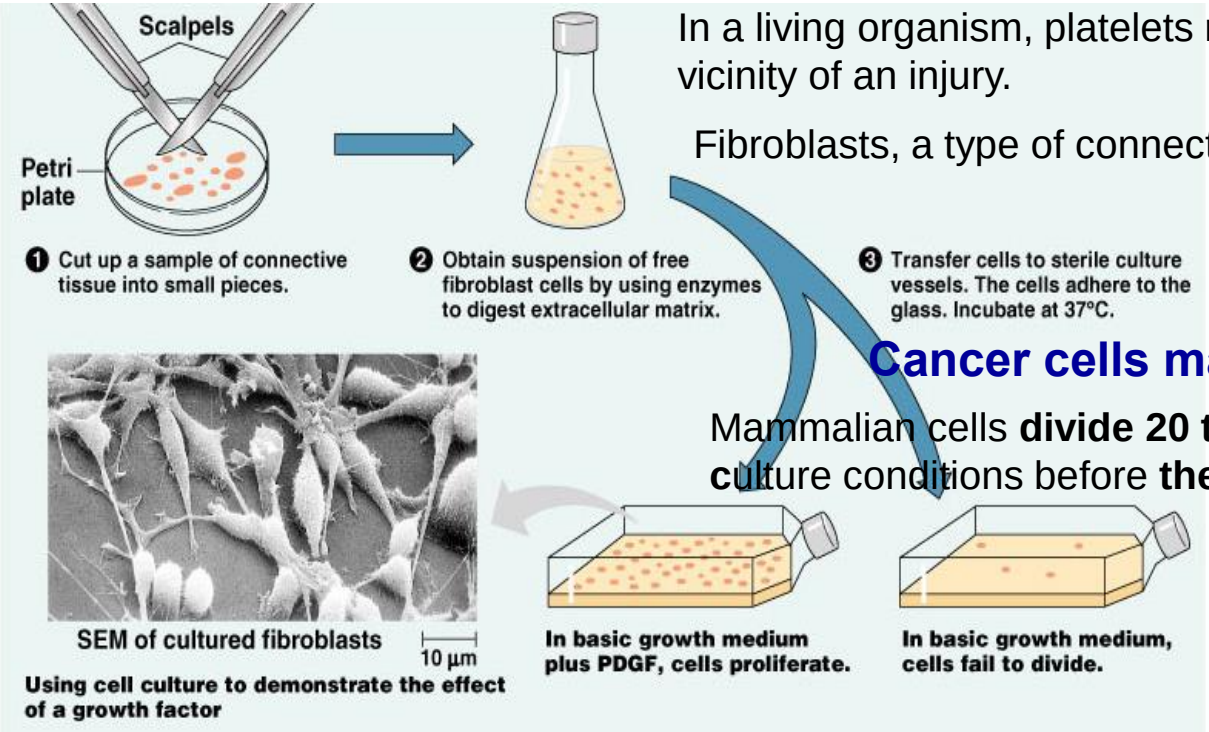
Cancer cells have escaped from cell cycle controls

- Cancer cells divide excessively and invade other tissues because they are free of the body's control mechanisms.
- If and when cancer cells stop dividing, they do so at random points, **not at the normal checkpoints** in the cell cycle.
- Cancer cell may divide indefinitely if they have a continual supply of nutrients.

Many external factors, both **chemical and physical,** that can influence the cell division

Growth factors: Cell division and cancer

– Stimulate other cells to divide



In a living organism, platelets release PDGF in the vicinity of an injury.

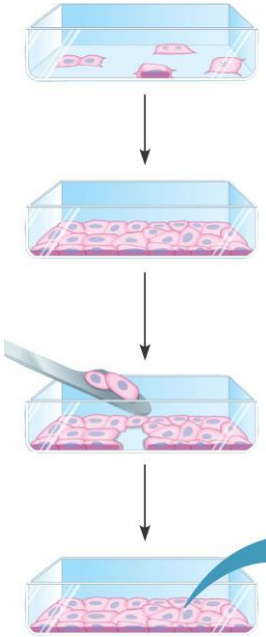
Fibroblasts, a type of connective tissue

Cancer cells may be "immortal".

Mammalian cells **divide 20 to 50 times under culture conditions before they stop, age, and die.**

Cancer cells do not stop dividing when growth factors are depleted either because they manufacture their own, have an abnormality in the signaling pathway, or have a problem in the cell cycle control system.

Density-dependent inhibition and anchorage dependence of cell division

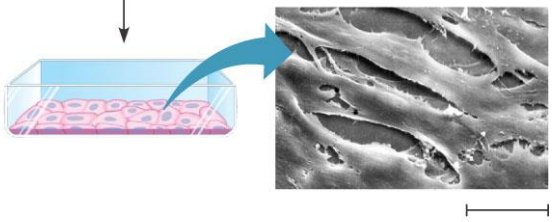


Cells anchor to dish surface and divide (anchorage dependence).

When cells have formed a complete single layer, they stop dividing (density-dependent inhibition).

If some cells are scraped away, the remaining cells divide to fill the gap and then stop (density-dependent inhibition).

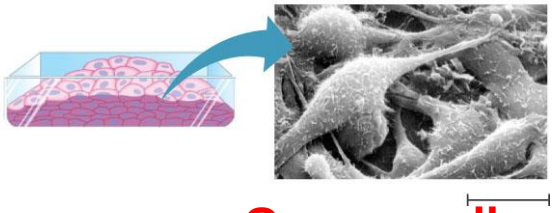
Normal mammalian cells. The availability of nutrients, growth factors, and a substratum for attachment limits cell density to a single layer.



Normal mammalian cells

Cancer cells

Exhibit neither density-dependent inhibition nor anchorage dependence



Cancer cells

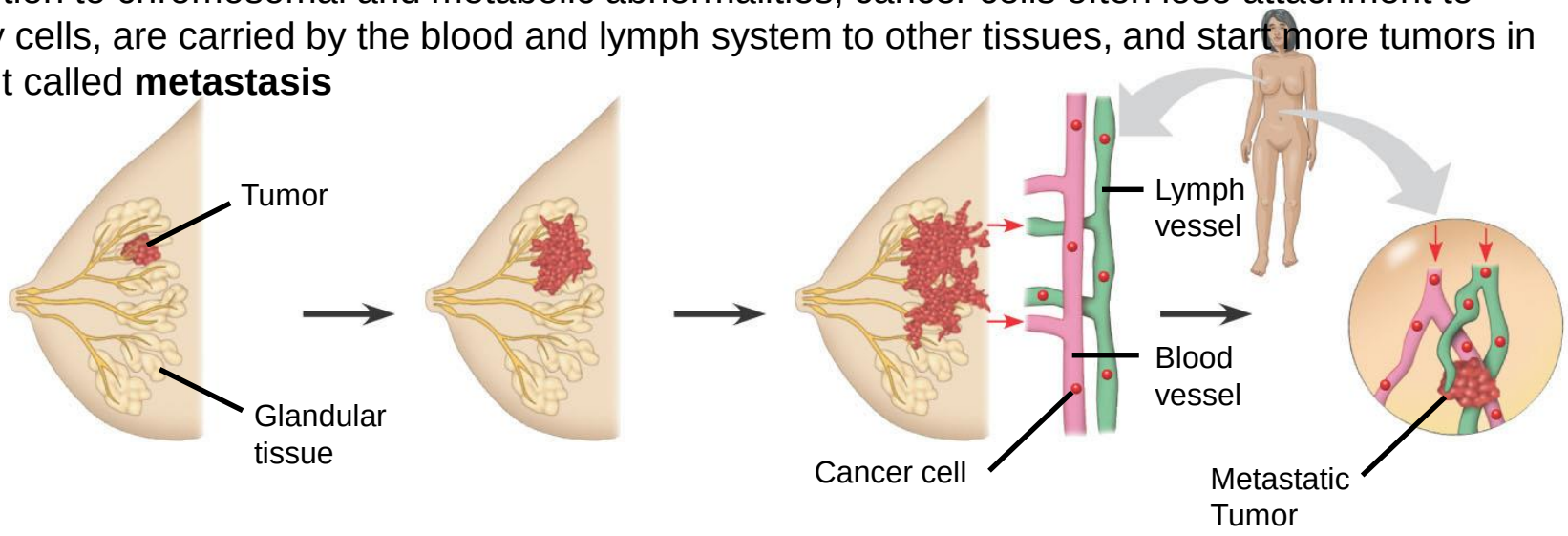
Cells (HeLa) from a tumor removed from a woman (Henrietta Lacks) in 1951 are still reproducing

Cancer cells have escaped from cell cycle controls

- Most animal cells also exhibit **anchorage dependence** for cell division.
- To divide they must be anchored to a substratum, typically the extracellular matrix of a tissue. Control appears to be mediated by connections between the extracellular matrix and plasma membrane proteins and cytoskeletal elements.
- The abnormal behavior of cancer cells begins when a single cell in a tissue undergoes a **transformation** that converts it from a normal cell to a cancer cell.
- Malignant tumors invade surrounding tissues and can metastasize: Exporting cancer cells to other parts of the body where they may form secondary tumors
- Cancer cells do not stop dividing when growth factors are depleted either because they manufacture their own, have an abnormality in the signaling pathway, or have a problem in the cell cycle control system.

Benign tumor to Malignant tumor

In addition to chromosomal and metabolic abnormalities, cancer cells often lose attachment to nearby cells, are carried by the blood and lymph system to other tissues, and start more tumors in a event called **metastasis**



Oncogene, Tumor-suppressor gene: Cell cycle and Cancer

•Cellular transformation (cancer cell formation) always involves the genes that somehow influence the cell cycle control system.

A **proto-oncogene** is a normal gene that can become an oncogene due to mutations or increased express.

An **oncogene** is a gene that has the potential to cause cancer. In tumor cells, they are often mutated or expressed at high levels

A **tumor suppressor gene**, or **antioncogene**, is a gene that protects a cell from one step on the path to cancer. Retinoblastoma protein and p53. Couple cell cycle to DNA damage response

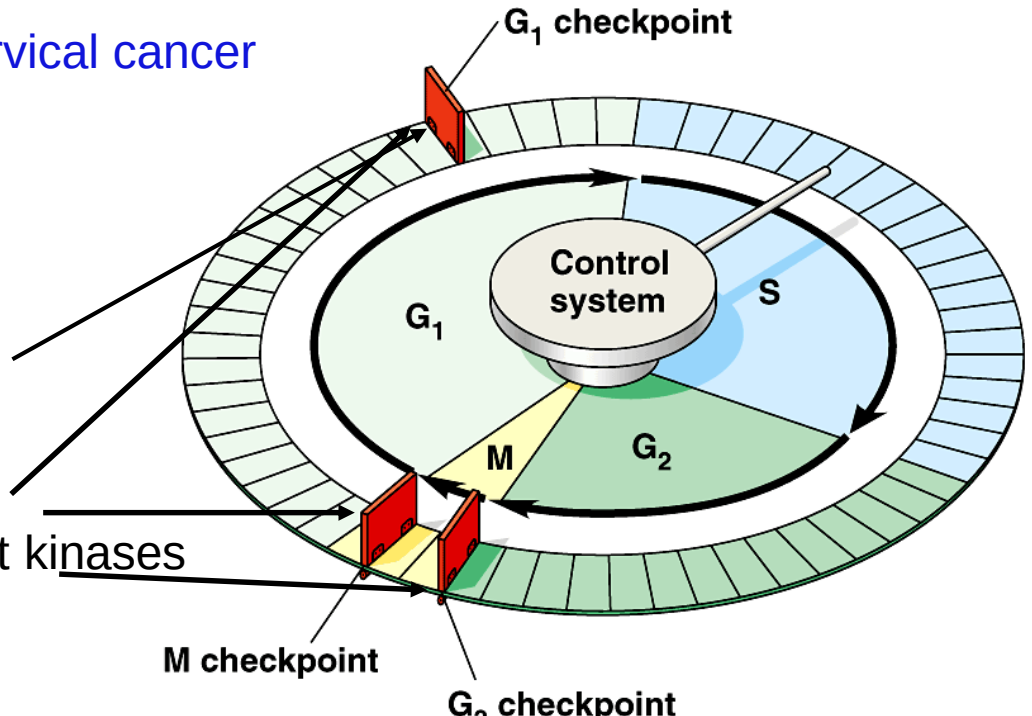
Human papiloma virus on p53: Cervical cancer

J. Michael Bishop and Harold E. Varmus: The Nobel Prize in Physiology or Medicine 1989: Retroviral-oncogene

Rb and p53

Hepatitis viruses: Liver cancer

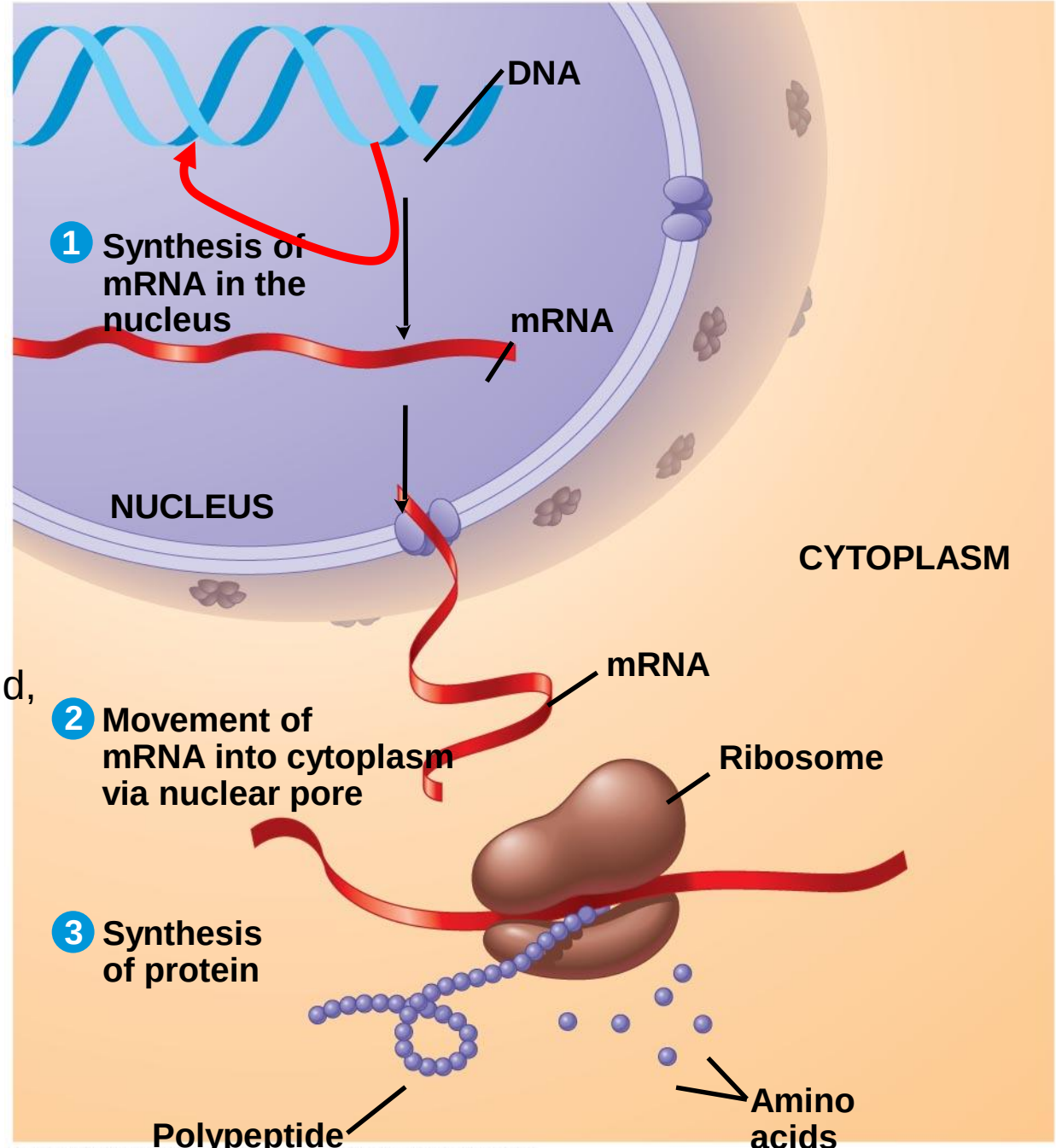
Raf Kinases and Cyclin Dependent kinases (Proto-oncogene)



Nucleic acids store and transmit hereditary information

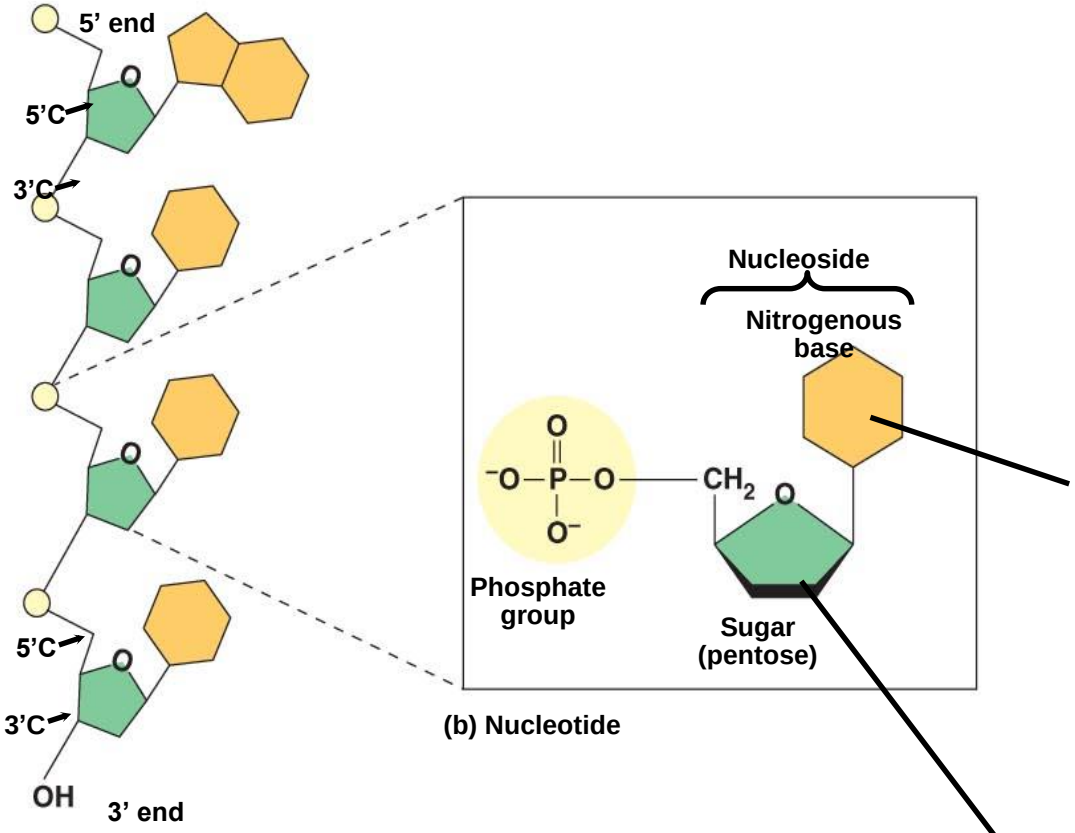
- **Genes** program the amino acid sequence of a polypeptide. Genes are made of DNA, a **nucleic acid**
- There are two types of nucleic acids: **Deoxyribonucleic acid (DNA); Ribonucleic acid (RNA)**
- DNA provides directions for its own replication

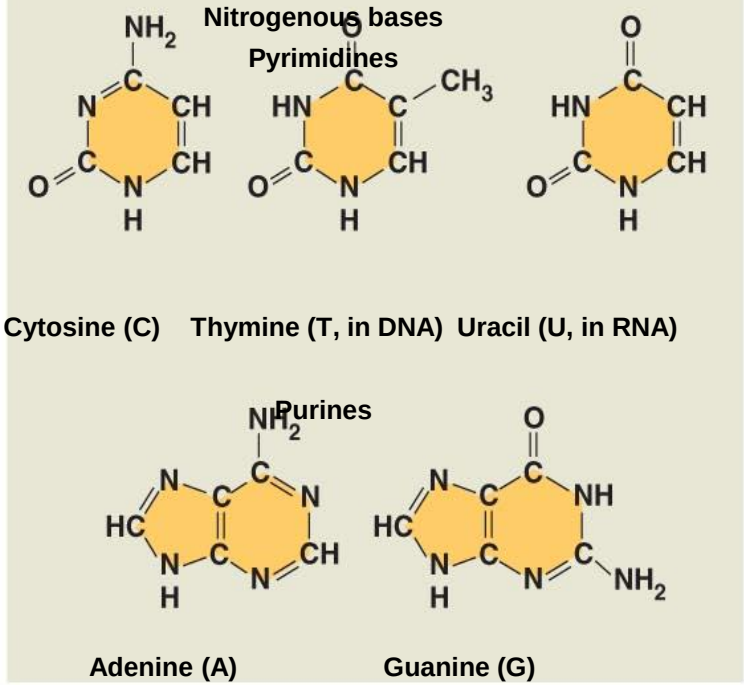
- DNA directs synthesis of messenger RNA (mRNA) and, through mRNA, controls protein synthesis
- Protein synthesis occurs in ribosomes

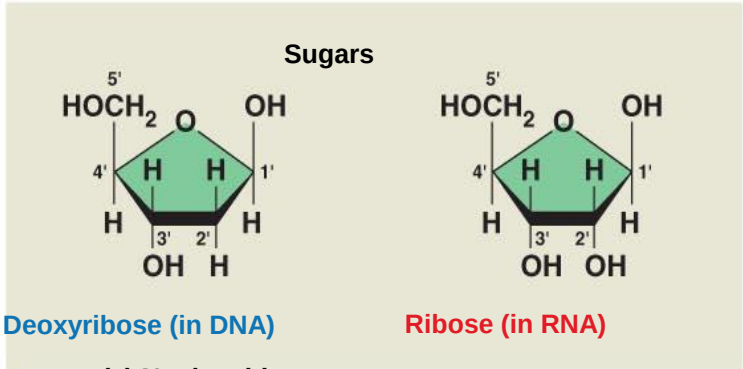


The Structure of Nucleic Acids

- Nucleic acids- polymers called **polynucleotides**
- Polynucleotides are made of monomers called **nucleotides** which consist of: nitrogenous base, pentose sugar and a phosphate group
- The sequence of bases (nucleotides) on DNA or mRNA polymers is unique to each gene



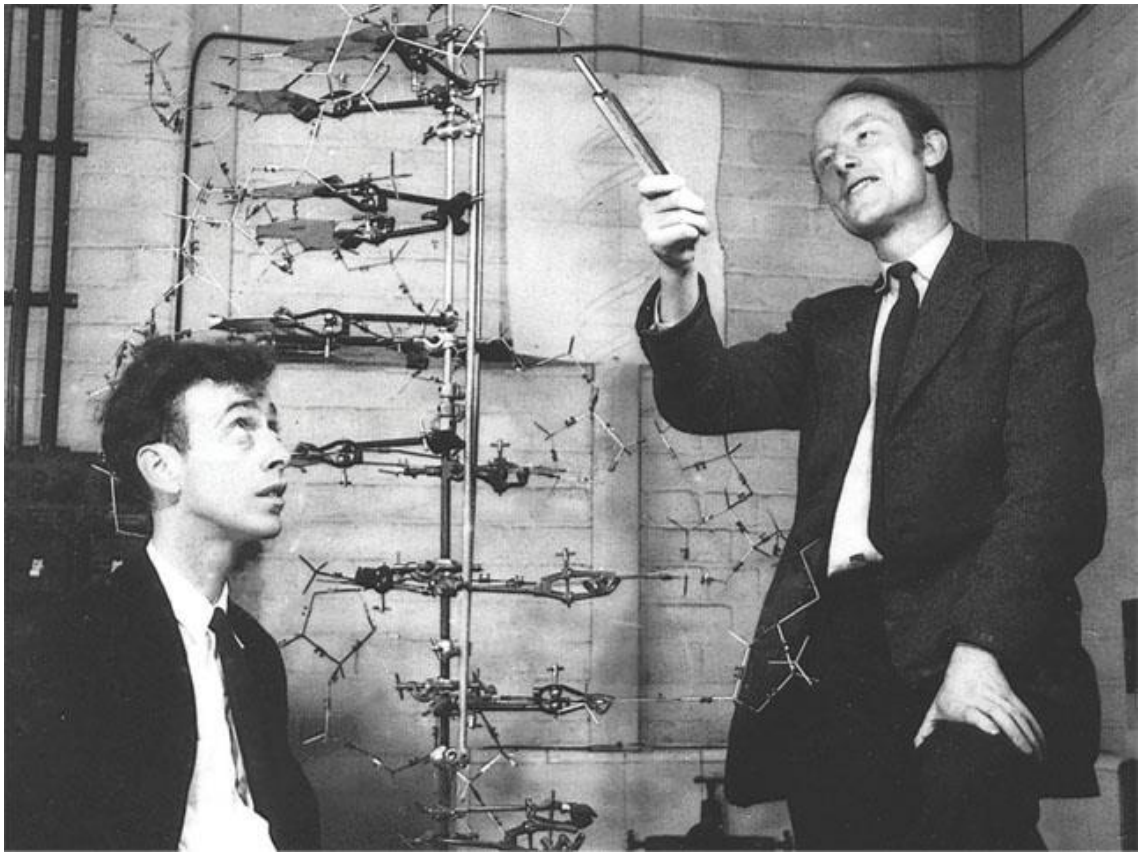




Nucleoside- a nucleotide lacking the phosphate group (base & sugar, only)
Nucleoside = nitrogenous base + sugar

Watson and Crick : DNA double helical structure

- Life's Operating Instructions
- In 1953, James Watson and Francis Crick shook the world

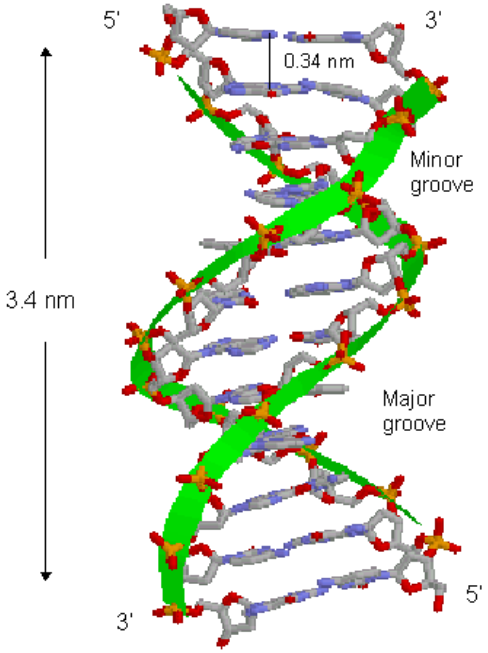


This **DNA** directs the development of **biochemical, anatomical, physiological, and behavioral traits**

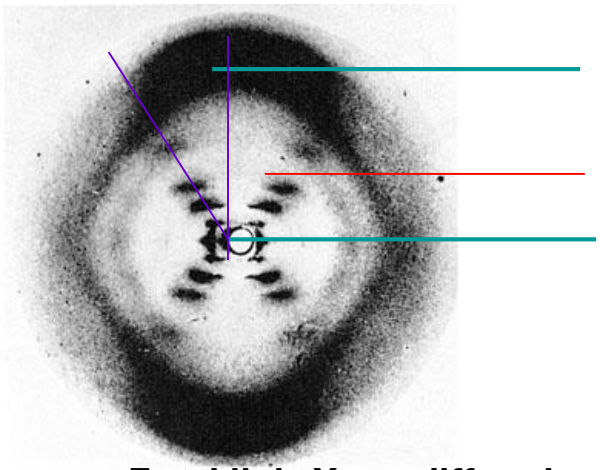
DNA structure answers mystery of possibility of **genetic instructions are held inside organisms and how information passed from generation to generation.**

Building a Structural Model of DNA

- Once most biologists were convinced that DNA was the genetic material
  - The challenge was to determine how the structure of DNA could account for its role in inheritance
- Maurice Wilkins and Rosalind Franklin
  - Were using a technique called X-ray crystallography to study molecular structure
- Rosalind Franklin
  - Produced a picture of the DNA molecule using this technique



(a) Rosalind Franklin



(b) Franklin's X-ray diffraction Photograph of DNA

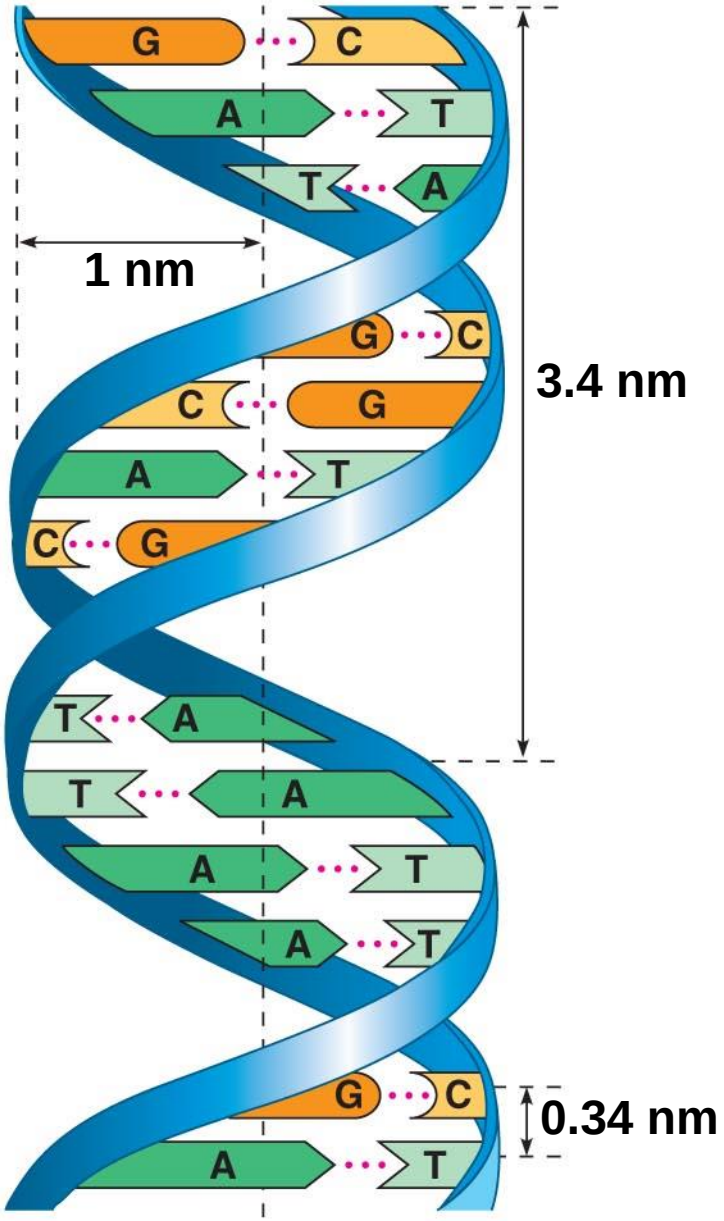
- Franklin had concluded that DNA
  - Was composed of two antiparallel sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior
- The nitrogenous bases
  - Are paired in specific combinations: adenine with thymine, and cytosine with guanine

•Chargaff's rules

In any organism the amount of A = T, and the amount of G = C or A + G = T + C

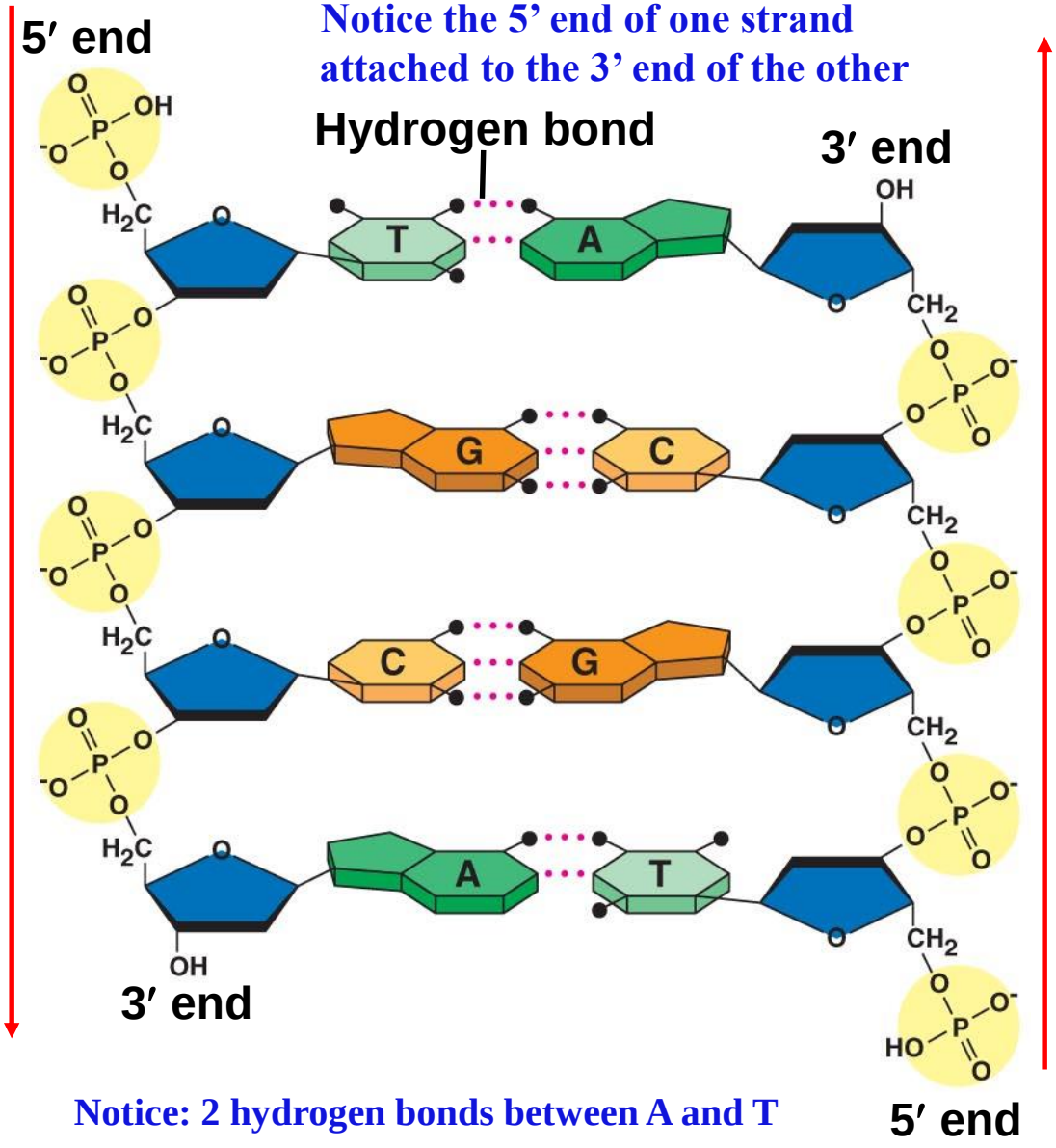
Franklin's X-ray crystallographic images to Watson and Crick's Double Helix structure

- Franklin's X-ray crystallographic images of DNA enabled Watson to deduce that DNA was helical
- The X-ray images also enabled Watson to deduce the width of the helix and the spacing of the nitrogenous bases
- The width suggested that the DNA molecule was made up of two strands, forming a **double helix**
- **The Nobel Prize in Physiology or Medicine 1962:** Francis Harry Compton **Crick**, James Dewey **Watson** and Maurice Hugh Frederick **Wilkins** "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material".



B-form DNA

(Right handed helix)



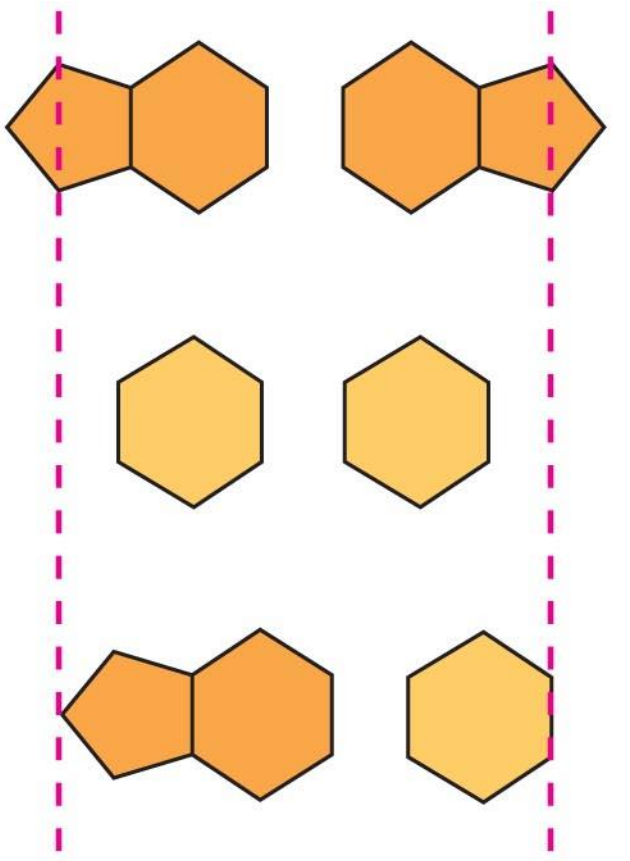
Notice: 2 hydrogen bonds between A and T

three hydrogen bonds between C and G

Directionality?

- At first, Watson and Crick thought the bases paired like with like (A with A, and so on), but such pairings did not result in a uniform width
- Watson and Crick reasoned that there must be additional specificity of pairing
  - Dictated by the structure of the bases
- Instead, pairing a purine with a pyrimidine resulted in a uniform width consistent with the X-ray (Purine: Adenine and Guanine; pyrimidine: Cytosine and Thymine)
- Each base pair forms a different number of hydrogen bonds
  - Adenine and thymine form two bonds, cytosine and guanine form three bonds

Uniform Geometry : Geometrical complementarity



Purine + purine: too wide

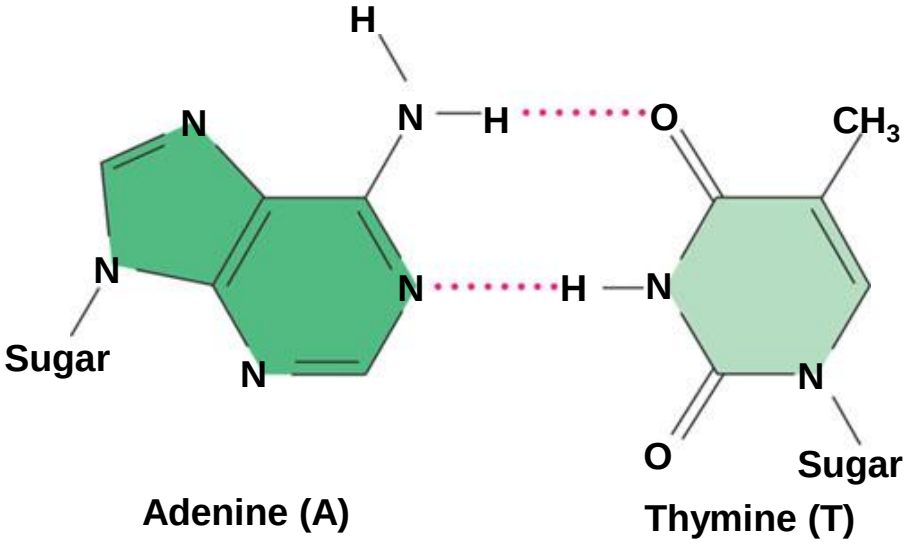
Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

Notice that a two ring structure (purine) bonds with a one ring structure pyrimidine

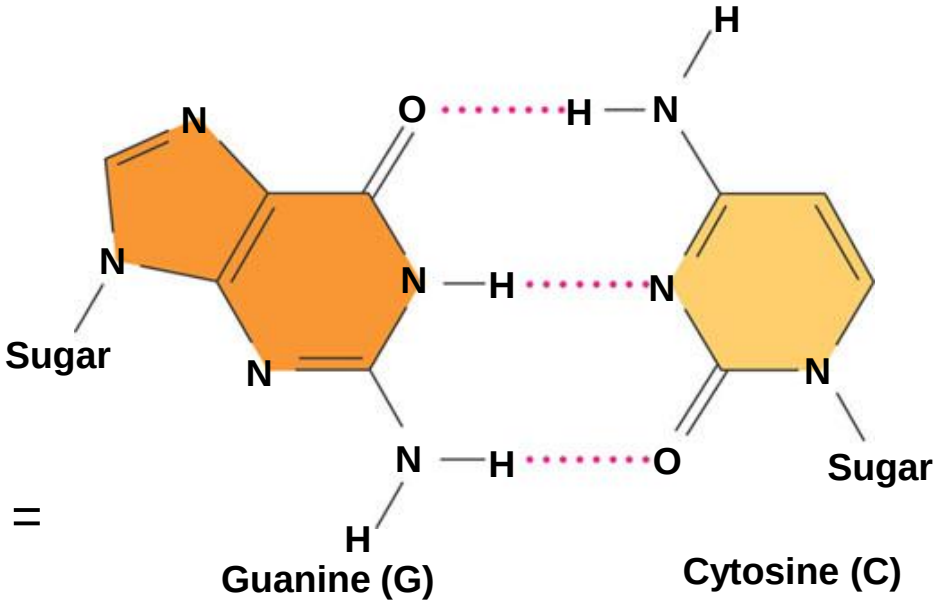
Chemical Complementarity

NH2: Partial Positive Charge
O : Partial negatively charged
N: Partial negative charge



Adenine (A) Thymine (T)

Miss pair A with C?



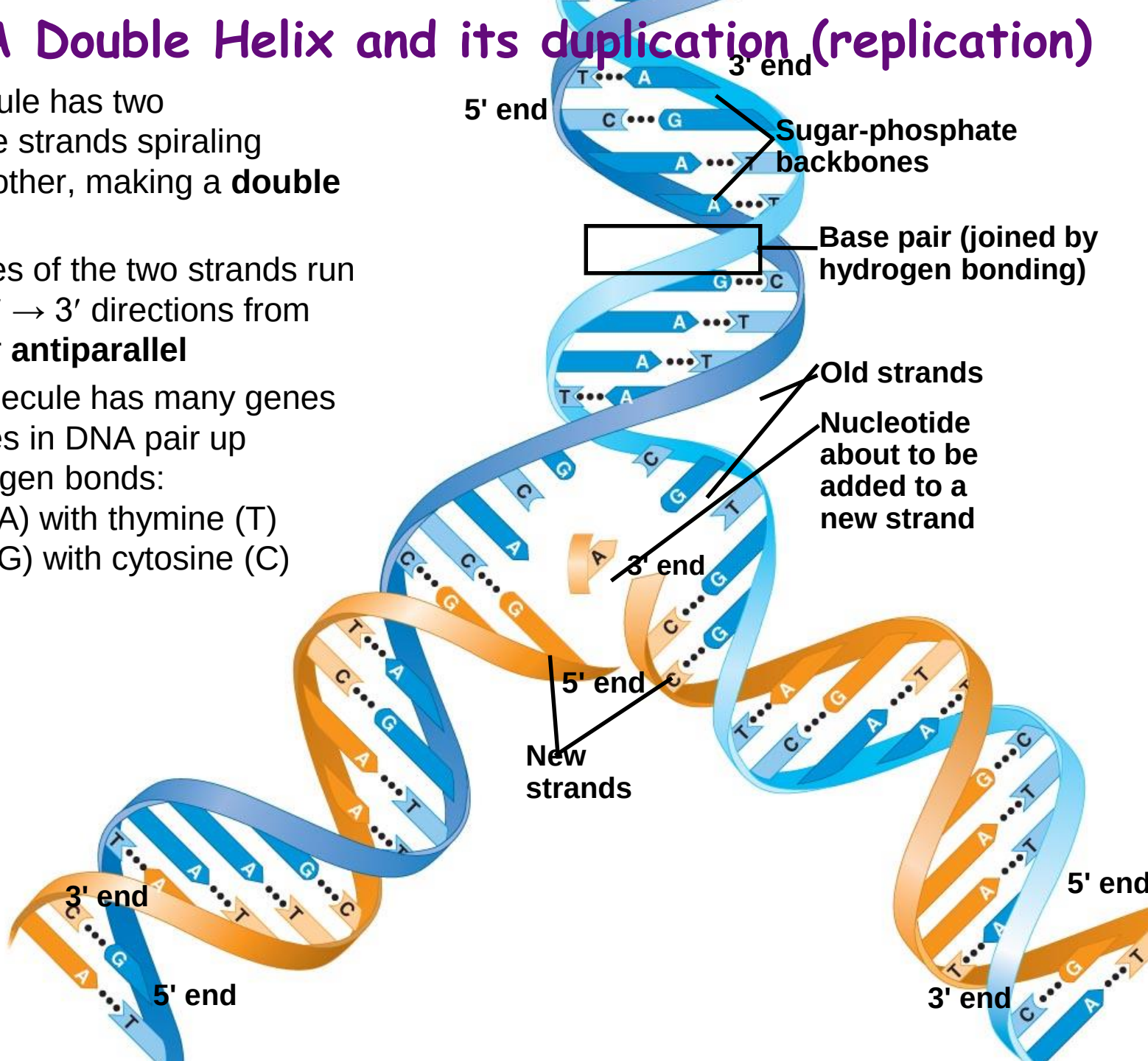
Guanine (G) Cytosine (C)

Chargaff's rules were answered:

In any organism the amount of A = T, and the amount of G = C

The DNA Double Helix and its duplication (replication)

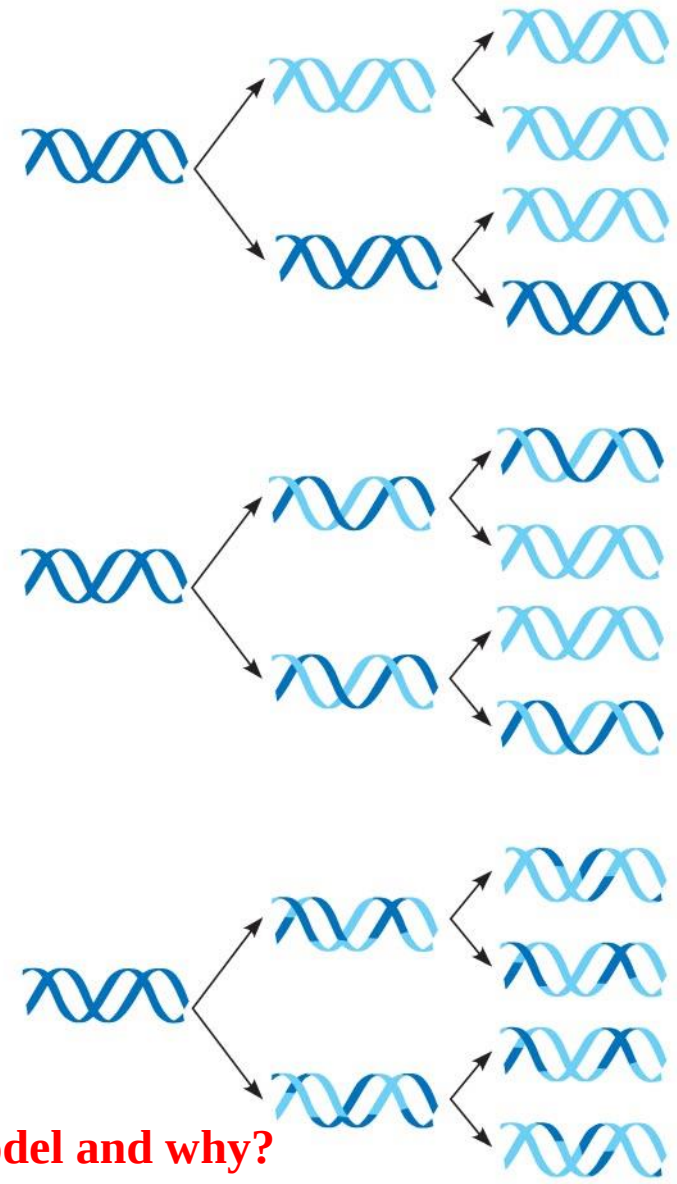
- A DNA molecule has two polynucleotide strands spiraling around each other, making a **double helix**
- The backbones of the two strands run in opposite 5′ → 3′ directions from each other, or **antiparallel**
- One DNA molecule has many genes
- Nitrogen bases in DNA pair up forming hydrogen bonds:
  - adenine (A) with thymine (T)
  - guanine (G) with cytosine (C)



The Basic Principle: Base Pairing to a Template Strand

- Since the two strands of DNA are complementary, each strand acts as a template for building a new strand in replication
- In DNA replication, the parent molecule unwinds, and two new daughter strands are built based on base-pairing rules

Models for DNA Replication



Conservative model. The two parental strands re-associate after acting as templates for new strands, thus restoring the parental double helix.

Semiconservative model. The two strands of the parental molecule separate, and each functions as a template for synthesis of a new, complementary strand.

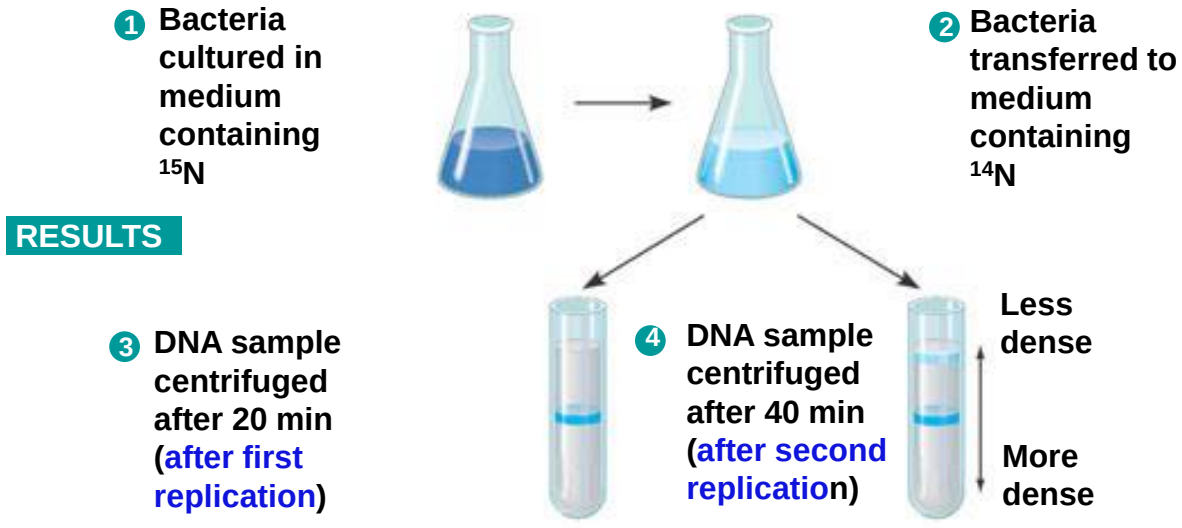
Dispersive model. Each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

Which is your favorite model and why?

Support for Semiconservative DNA Replication

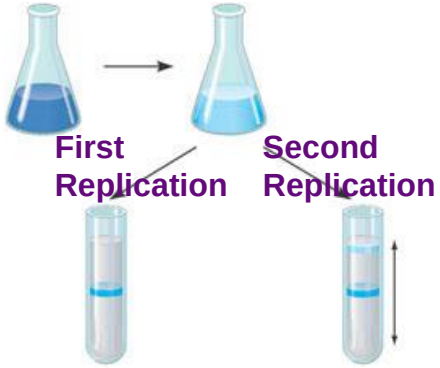
Meselson and Stahl Experiments

- Cultured *E. coli* bacteria for several generations on a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N.
- The bacteria incorporated the heavy nitrogen into their DNA. The scientists then transferred the bacteria to a medium with only ^{14}N, the lighter, more common isotope of nitrogen.
- Any new DNA that the bacteria synthesized would be lighter than the parental DNA made in the ^{15}N medium.
- Meselson and Stahl could distinguish DNA of different densities by centrifuging DNA extracted from the bacteria.



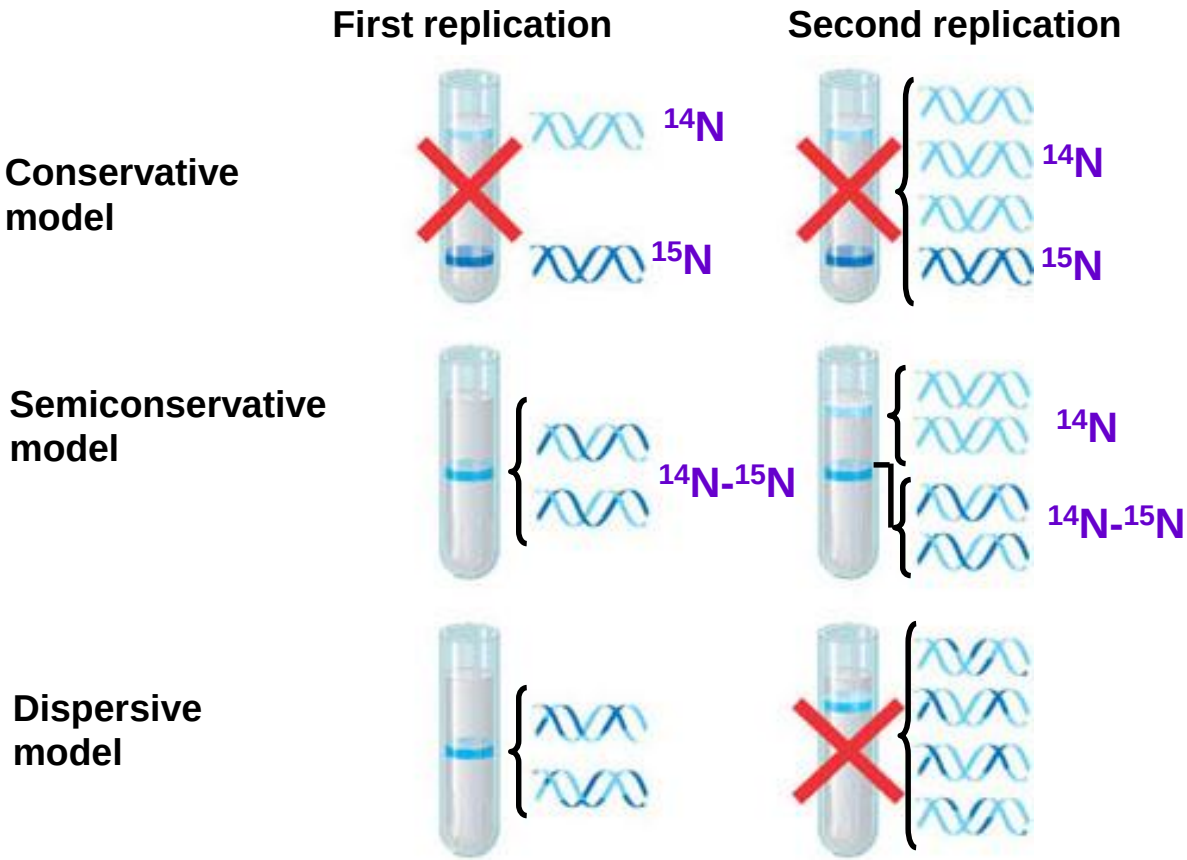
Conclusion: DNA replication is semi conservative

Conclusion



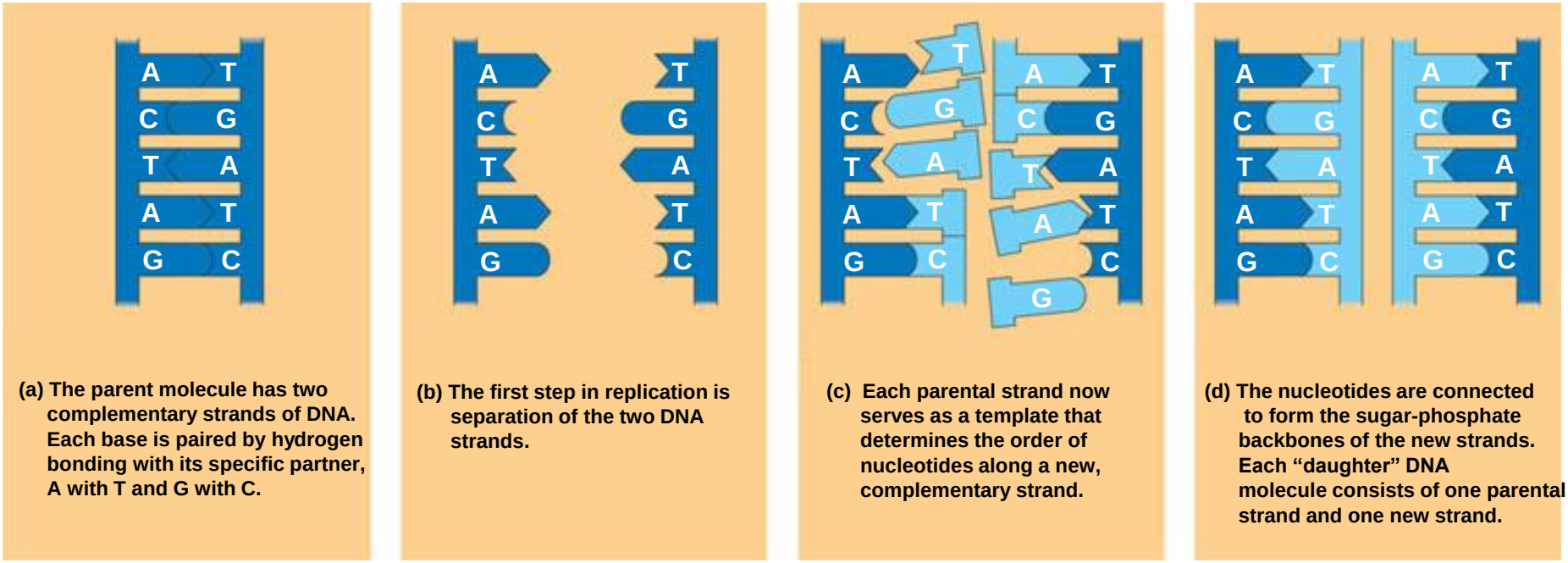
Results

Interpretation (Expected results)



Conclusion: DNA replication is semi conservative

- In DNA replication
  - The parent molecule unwinds, and two new daughter strands are built based on base-pairing rules



(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.

(c) Each parental strand now serves as a template that determines the order of nucleotides along a new, complementary strand.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

- The copying mechanism is analogous to **using a photographic negative to make a positive image, which can in turn be used to make another negative, and so on.**