BIO 311C
Spring 2010

Your graded Exam 3 will be available for you to pick up at the end of today's lecture.

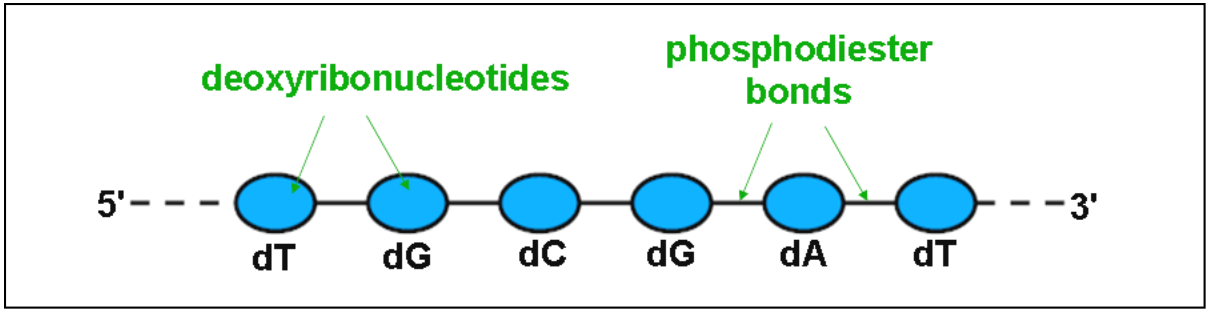
The key to Exam 3 has been posted on the course web site on Wednesday (April 21).

If you believe that a question on your Exam 3 was graded incorrectly or your score was calculated inaccurately, then see Rebecca before 4:00 p.m. on Monday (April 26) to explain your concern. We cannot consider changes after that time.

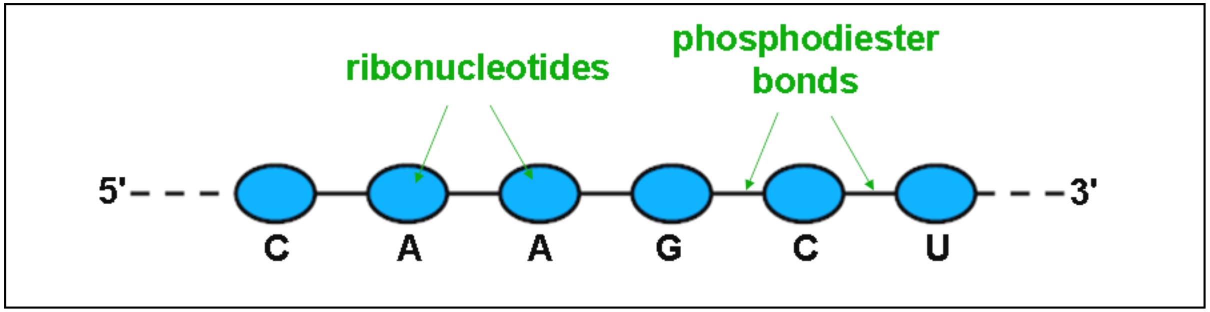
Jerry Brand will not be available for Office Hours on Thursday April 23).

Lecture 33 – Wednesday 21 Apr.

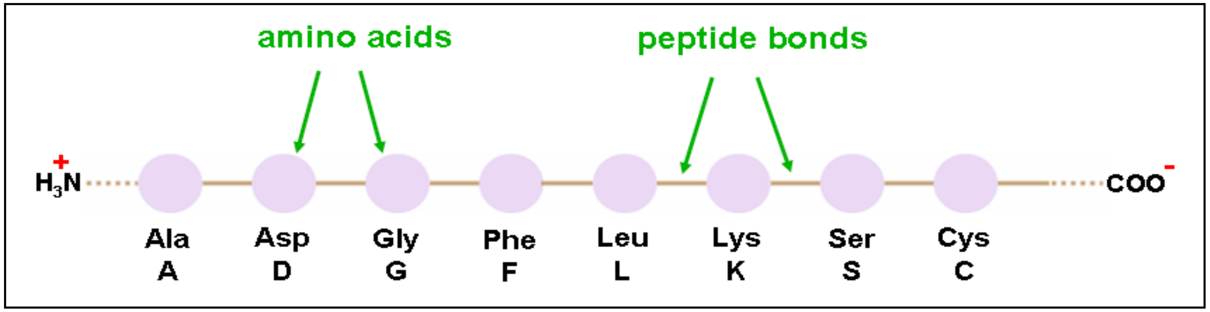
Definition: Bioinformation molecule - a biopolymer whose sequence of monomers conveys useful information in the cell. The three kinds of information molecules in cells are DNA, RNA and polypeptides.



Primary Structure of a portion of a strand of DNA



Primary structure of a portion of a molecule of RNA



Primary structure of a portion of a polypeptide chain

The information content of bioinformation molecules resides in their primary structures.

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Major roles of Bioinformation Molecules in Cells

DNA

long-term storage of information

RNA

short-term storage of information,
regulation of expression of information,
management and conveyance of information

Protein (polypeptide chains)

expression of information

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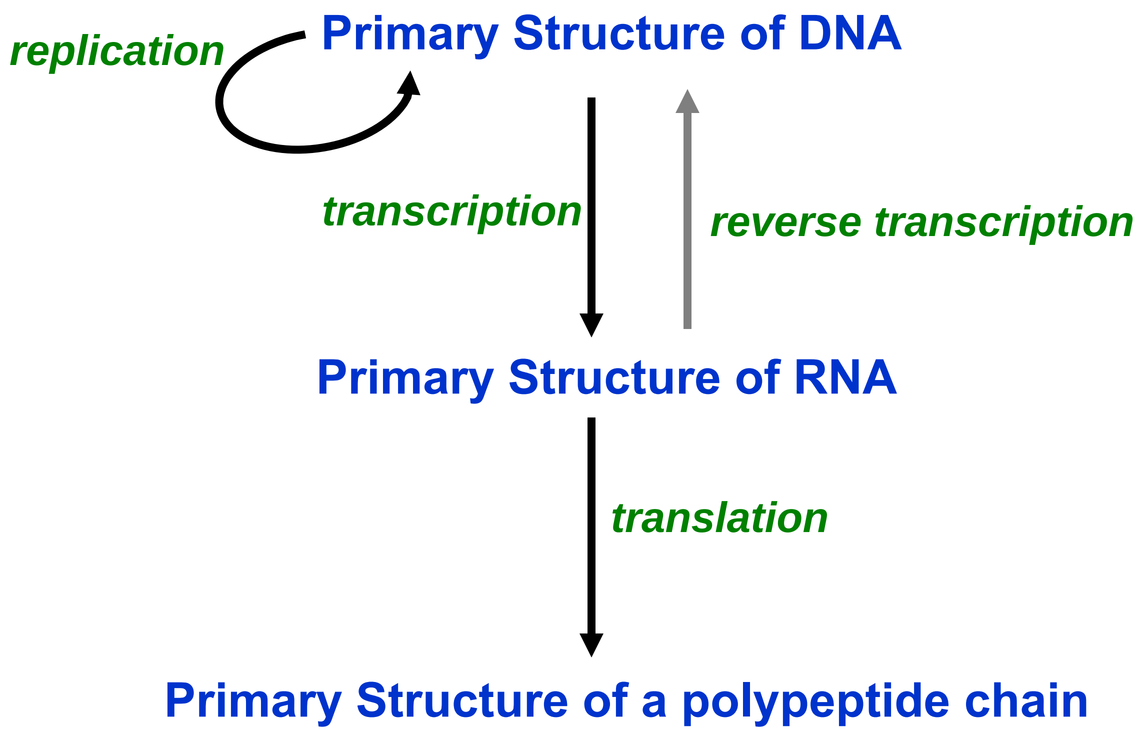
Information Flow in Cells



This sequence of events is often called the "central dogma" of molecular biology.

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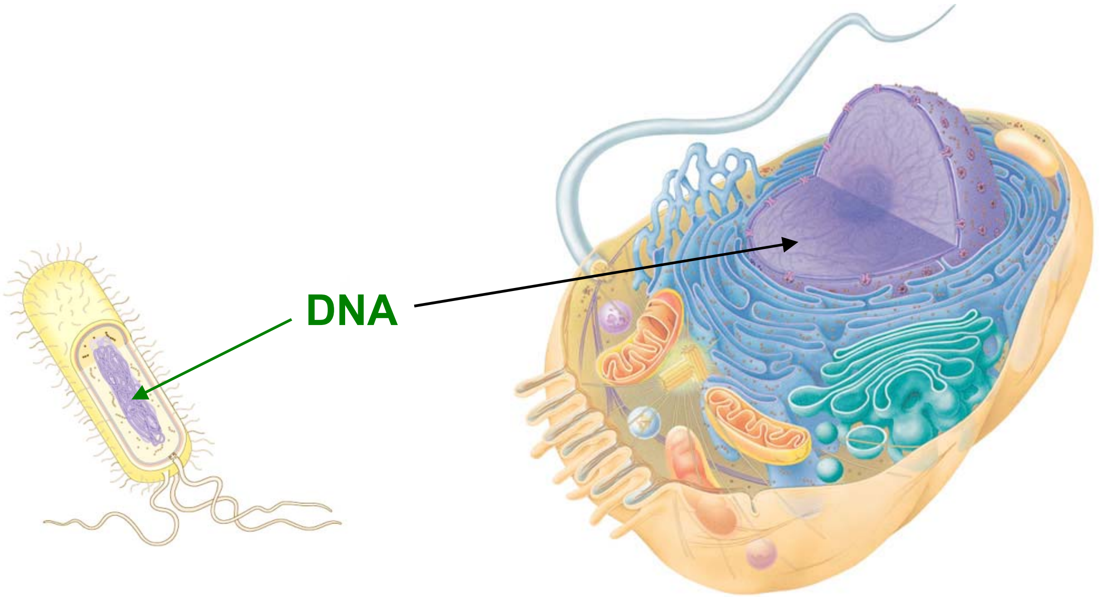
DNA is an essential component of all living cells.

DNA resides in the cytoplasmic matrix of prokaryotic cells.

Most DNA resides in nucleus of eukaryotic cells, with a small amount also in mitochondria and plastids.

DNA does not move from one compartment to another in cells under normal circumstances.



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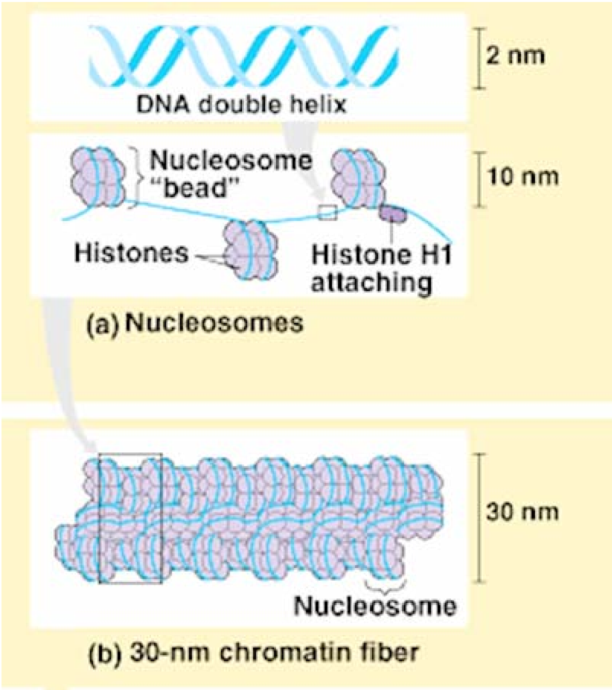
DNA occurs in cells as a very long double helix of two polynucleotide chains.

The double helix of DNA in eukaryotes is organized in cells by binding to, and wrapping around, specific proteins called histones.

This binding and wrapping in eukaryotic cells is very elaborate and organized, producing a complex structure called chromatin. Chromatin contains approximately 50% DNA and 50% protein.

chromatin packing in eukaryotic cells



Typical eukaryotic cells contain hundreds of times more DNA per cell than do typical prokaryotic cells.

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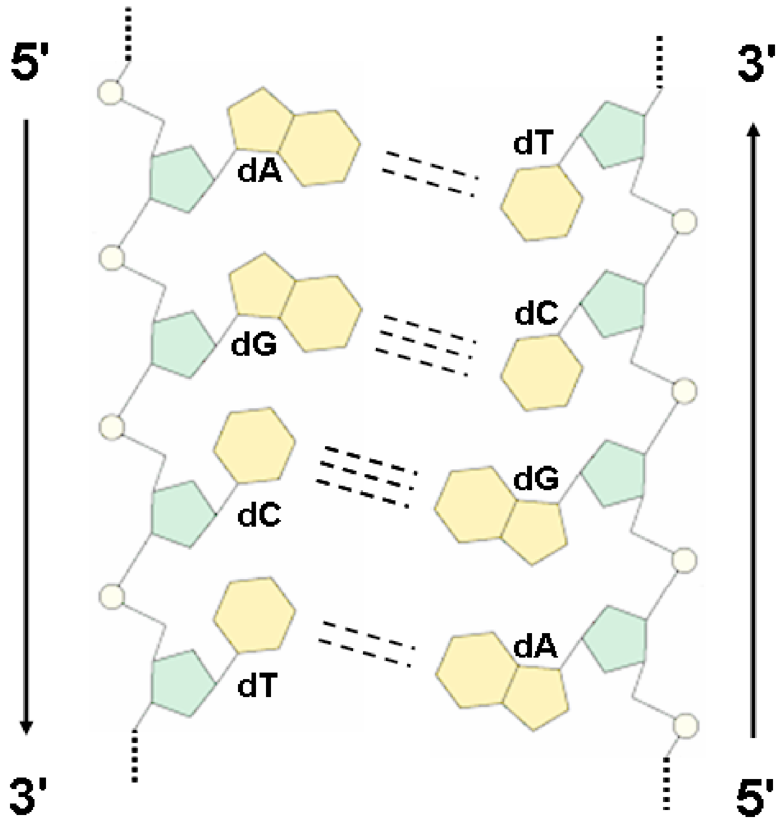
- The polynucleotide chains of DNA are antiparallel to each other.

- The nucleoside dA is always directly across from the nucleoside dT.
  The nucleoside dG is always directly across from the nucleoside dC.

- Each adjacent pair of dA and dT is hydrogen-bonded by two H-bonds.
  Each adjacent pair of dG and dC is hydrogen bonded by three H-bonds.



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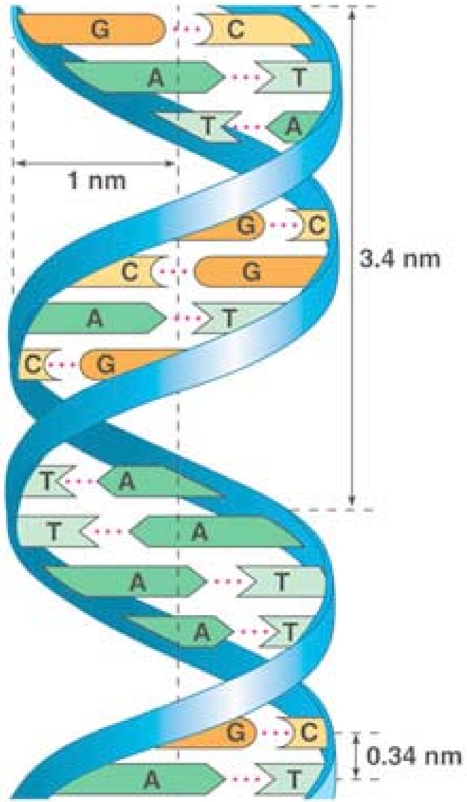
The Double-helix of DNA
Showing Complementary base Pairing



Textbook Fig. 16.7a

The two strands of a DNA double helix are referred to as "complementary strands".

Two bases that are directly adjacent to each other and hydrogen-bonded together in a DNA double helix are called a complementary base pair.

Molecules of DNA are very thin, approximately 2 nm in diameter.

There are 10 nucleotide-pairs for every complete rotation of a molecule of DNA

Molecules of DNA extend 3.4 nm in length for each full rotation.

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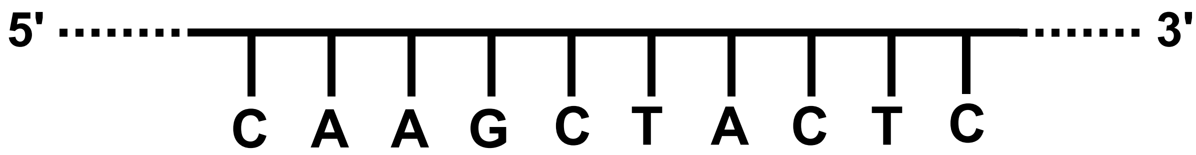
Simplified illustration of a portion of one strand of a DNA molecule



note: the nucleotides are shown here as A, T, G and C rather than the more accurate designations as dA, dT, dG and dC. The "d" prefix is generally omitted when it is understood that the represented molecule is DNA rather than RNA.

What feature of the molecule represented in this illustration indicates that it is a strand of DNA rather than a strand of RNA?

You should be able to draw the complementary strand of DNA next to this strand, showing:

- complementary base pairing,
- the 5' and 3' ends of the complementary strand,
- the number of hydrogen bonds between each base pair.

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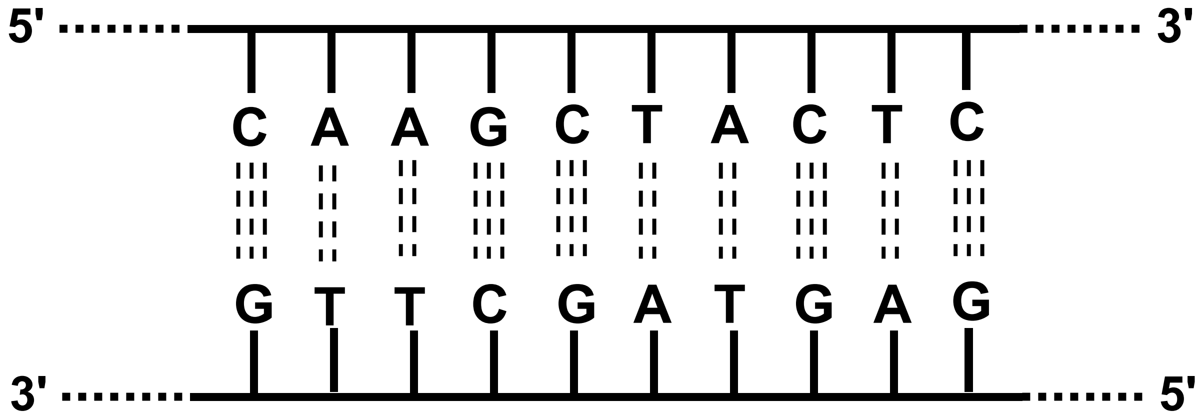
Illustration of a portion of a molecule of DNA



More abbreviated illustration of the same portion of the molecule

5' .... C A A G C T A C T C .... 3'

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The size of a molecule of DNA is often given as its number of complementary base pairs or kilobase pairs.

The molecule of DNA in a typical prokaryotic cell is a few million base-pairs long. For example, the chromosome of *E. coli* is approximately 4.6 million base pairs (4.6×10^6 bp), or 4,600 kilobase pairs (4.6×10^3 kb).

The average length of a molecule of DNA in a unit of chromatin (a chromosome) in a human cell is nearly 150 million ($1.5 \text{ X } 10^8$) bp = $1.5 \text{ X } 10^5$ kb.

Human cell nuclei contain 46 units of chromatin, each of which becomes compacted into a chromosome during cell division.

Thus, the total amount of DNA in a human cell nucleus is approximately 6.6 billion (= 6.6×10^9) bp.
= 6.6×10^6 kb.

A molecule of DNA in a typical animal mitochondrion is approximately 16 kb.

A molecule of DNA in a typical plastid is approximately 150 kb.

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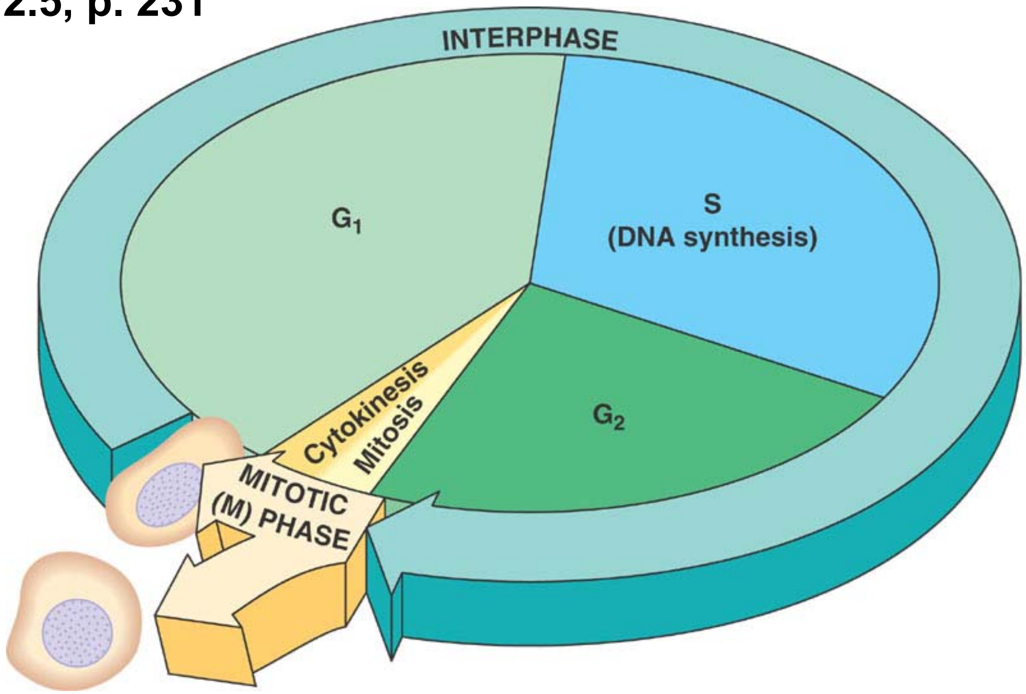
DNA synthesis is only one stage (phase) in the life of a cell.

The Cell Cycle of a Typical Replicating Eukaryotic Cell

Textbook fig. 12.5, p. 231



Chapter 12 is not assigned reading for this semester, but you should know the different stages of the cell cycle (this Fig.) and the general features of each stage (shown on the following presentation slide).

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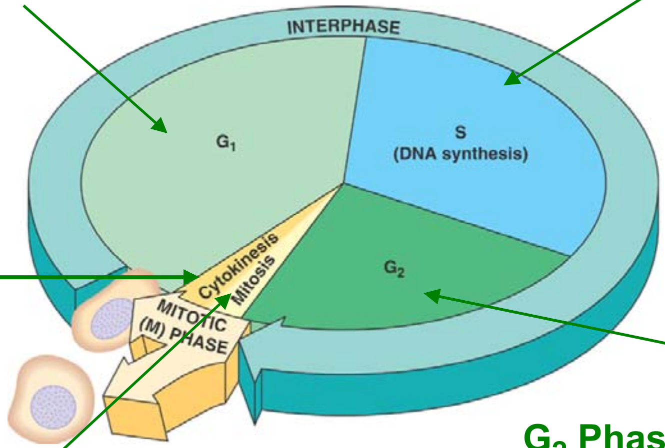
Phases of the Cell Cycle of Eukaryotic Cells

G_1 Phase: The recently divided cell
(1) grows in size
(2) performs routine metabolic activities
(3) makes an irreversible decision whether to prepare to divide again or else to mature and begin performing its mature functions

S Phase: if the cell has made the irreversible decision to divide, then its DNA is replicated. Cells that instead mature, do not go into an S phase.

Cytokinesis = cell division



G_2 Phase: If the cell has made the irreversible decision in G_1 to divide, and its DNA has replicated in S, then during the subsequent G_2 phase much of the cell's metabolism is diverted to preparation for the M Phase. If the cell instead will begin to perform mature functions, then it continues with its mature functions in a continuous G phase.

M Phase: If the cell has prepared to divide in G_2, then it performs:
(1) mitosis
(2) cytokinesis (cell division)

Most events in the cell cycle are not visible by light microscopy, but M-phase activities are easily visible.

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Definition - DNA Replication: The process used by cells to duplicate a molecule of DNA such that the two molecules have identical primary structures (sequence of nucleotides).

Three sequential events involved in reproduction of eukaryotic cells should not be confused:

- Replication (of DNA)

- Mitosis (movement of the two copies of DNA to different parts of the cell)

- Cytokinesis (cell division)

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Some Potential Problems with DNA Replication

- **The cell must replicate millions of nucleotides in a molecule of DNA over a very short period of time.**

  For example, the bacterium E. coli can replicate every 20 minutes. Thus, it must replicate the 4 million nucleotides in its chromosomes in less than 20 minutes.

- **The cell replication machinery must reproduce extremely long molecules of DNA without tangling or breaking the molecules.**

  For example, DNA molecules are only ~2 nm in diameter, yet some DNA molecules in humans are over an inch (2.5 cm) long. Very long and thin molecules are especially susceptible to breaking.

- **The DNA must be replicated with nearly 100% fidelity.**

  i.e. There can not be any mistakes in replicating the sequence of DNA or else the genetic information will be altered permanently in the new DNA and in the DNA of all subsequent generations.

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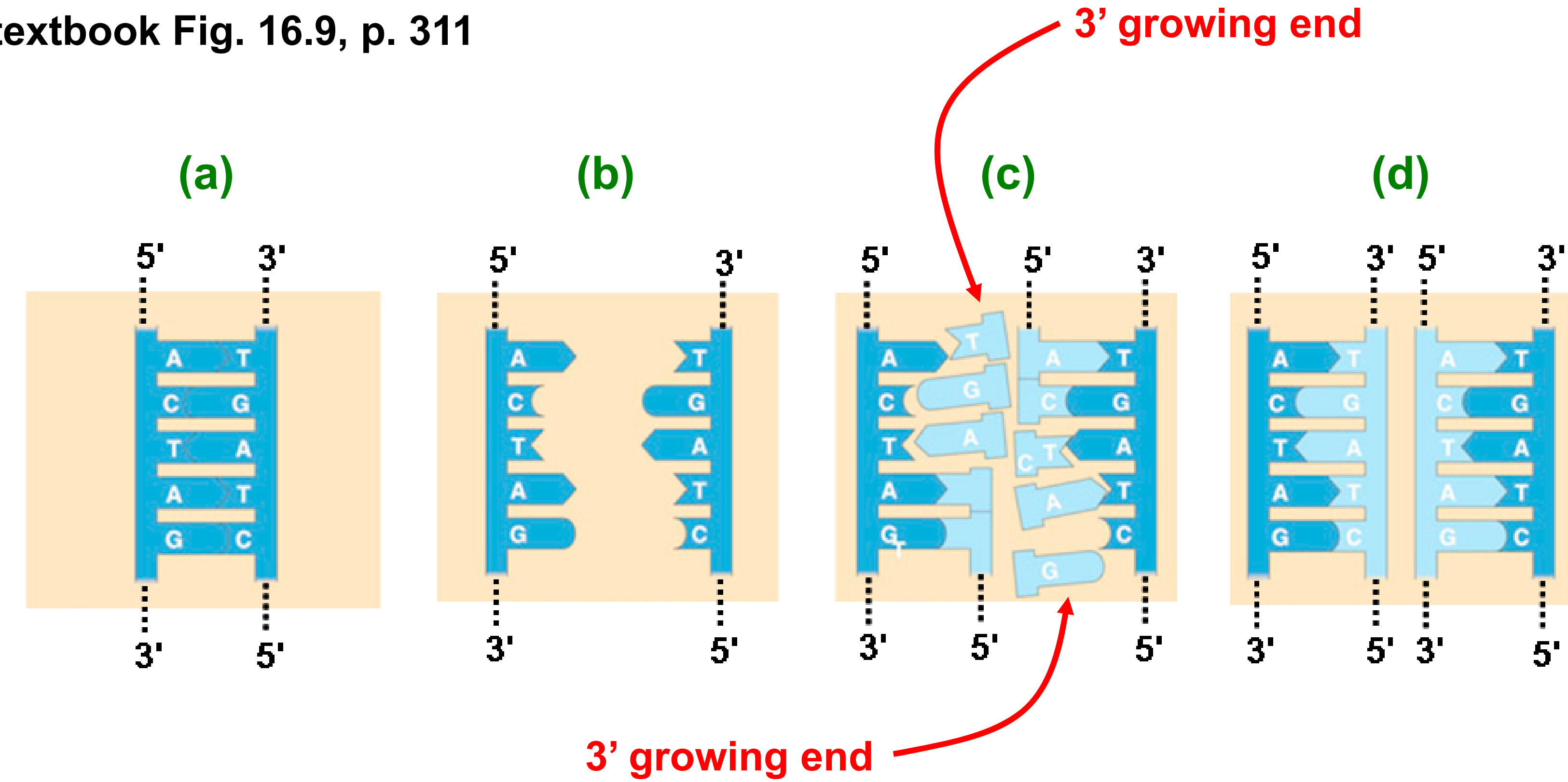
DNA is replicated by a semiconservative process.
New polynucleotide chains are extended in the 5'-to-3' direction.

From textbook Fig. 16.9, p. 311



During replication of a molecule of DNA, each polynucleotide chain remains intact, and each serves as a template for the construction of a new complementary polynucleotide chain.

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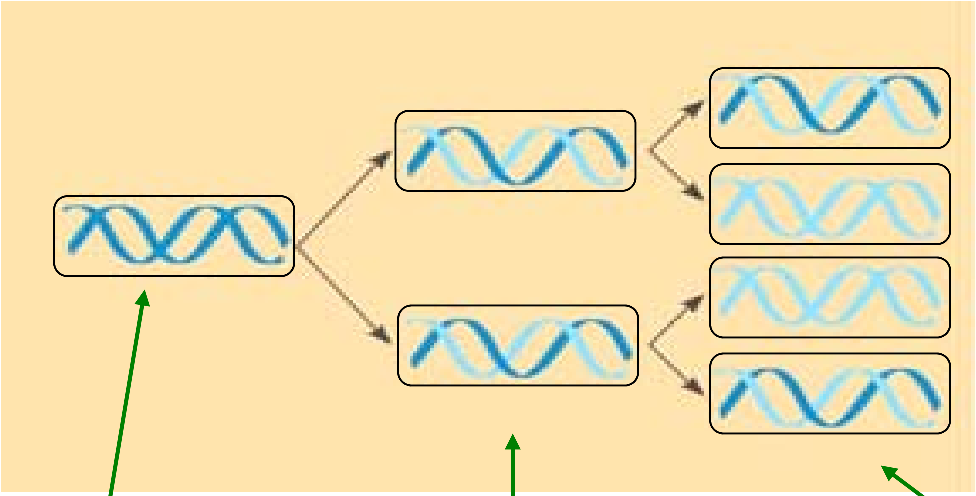
Each new polynucleotide chain is antiparallel to its template molecule.

The semiconservative mechanism of DNA replication maintains intact strands of DNA through successive generations of cells.

Modified from textbook Fig. 16.10b, p. 312



DNA molecule in a cell

DNA molecule in each of four cells resulting from cell divisions of the two cells at left

DNA molecule in each of two cells resulting from the cell division of the cell at left

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The Central enzyme in DNA replication is DNA Polymerase.

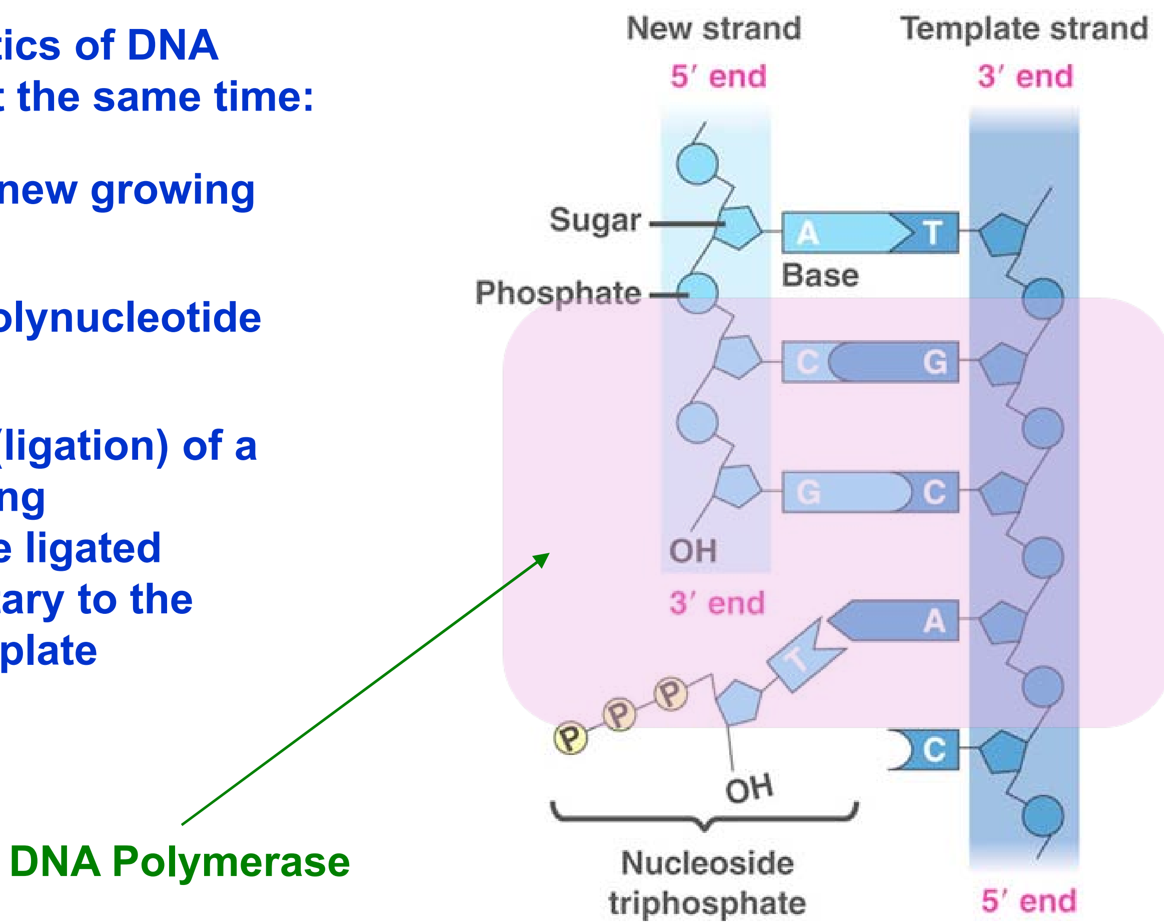
From Textbook Fig. 16.14, p. 315

Three functional characteristics of DNA Polymerase are expressed at the same time:

(1) it binds to the 3' end of a new growing polynucleotide chain,

(2) it binds to the template polynucleotide chain,

(3) it catalyzes the insertion (ligation) of a nucleotide into the growing polynucleotide chain. The ligated nucleotide is complimentary to the adjacent base on the template polynucleotide chain.



Several different DNA polymerases occur in eukaryotic cells. The DNA polymerase that is central to the insertion of deoxyribonucleotides into the growing polypeptide chain during the S phase is called pol III.

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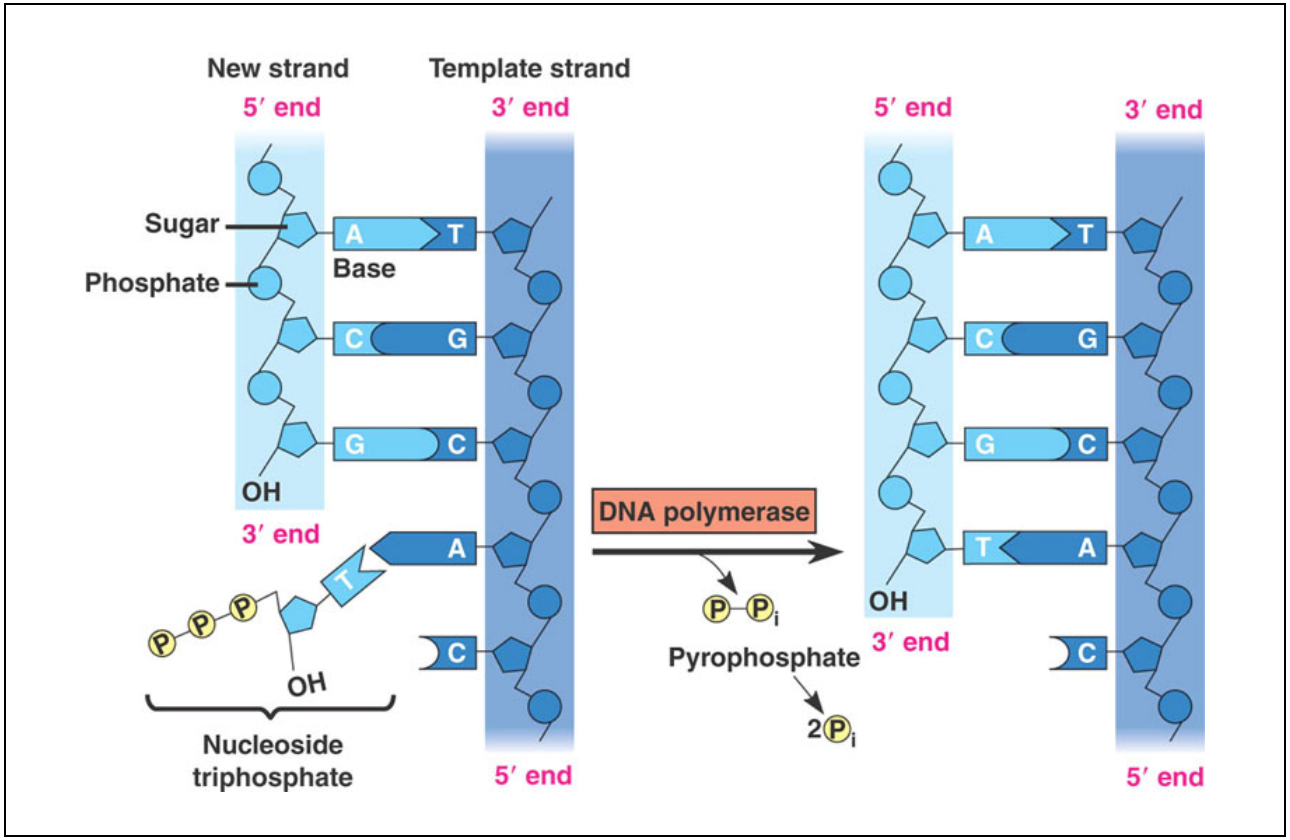
Reaction Catalyzed by DNA Polymerase



Textbook Fig. 16.14, p. 315

Note: this reaction extends the growing polynucleotide chain by one nucleotide in the 5'→3' direction.

pyrophosphate

$$(\text{polynucleotide})_n + \text{dNTP} \xrightarrow{\textit{DNA polymerase}} (\text{polynucleotide})_{n+1} + \text{P}\sim\text{P}$$

A second reaction coupled to this reaction is:

$$\text{P}\sim\text{P} + H_2O \xrightarrow{\textit{pyrophosphatase}} 2\ P_i$$

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Energetics of the Ligase Function of DNA Polymerase

1. $(\text{polynucleotide})_n + \text{dNTP} \xrightarrow{\textit{DNA polymerase}} (\text{polynucleotide})_{n+1} + \text{P}\sim\text{P}$

$\Delta G° \approx -15$ kJ/mole

2. $\text{P}\sim\text{P} + H_2O \xrightarrow{\textit{pyrophosphatase}} 2\ P_i$

$\Delta G° \approx -30$ kJ/mole

Sum: $(\text{polynucleotide})_n + \text{dNTP} + H_2O \xrightarrow[\textit{pyrophosphatase}]{\textit{DNA polymerase}} (\text{polynucleotide})_{n+1} + 2\ P_i$

$\Delta G° \approx -45$ kJ/mole

Thus, ≈ - 45 kJ/mole of energy is released for each nucleotide inserted into the growing polynucleotide chain, making the ligation reaction virtually irreversible.

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