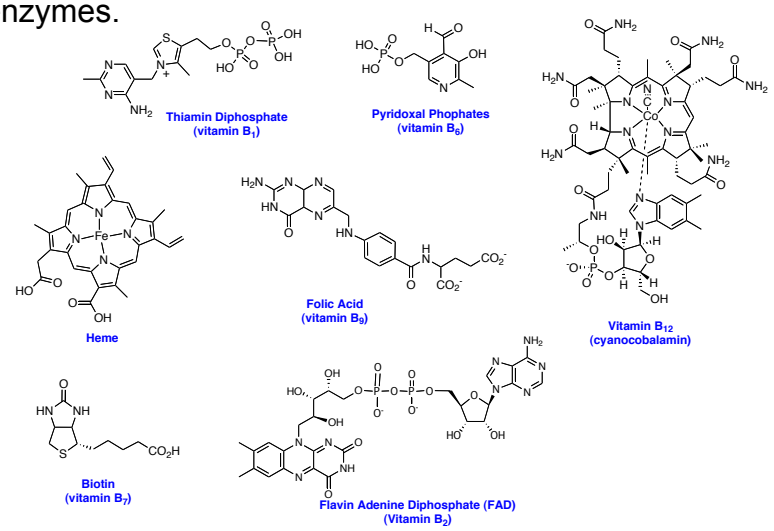
25.19: Coenzymes. Some reactions require additional organic molecules or metal ions. These are referred to as cofactors or coenzymes.



25.20: Protein Quaternary Structure: Hemoglobin. (please read)
25.21: G-Coupled Protein Receptors. (please read)

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Chapter 26: Nucleosides, Nucleotides, and Nucleic Acids. Nucleic acids are the third class of biopolymers (polysaccharides and proteins being the others).

Two major classes of nucleic acids:
deoxyribonucleic acid (DNA): carrier of genetic information.

ribonucleic acid (RNA): an intermediate in the expression of genetic information and other diverse roles.

The Central Dogma (F. Crick):

$$\underset{\text{(genome)}}{\text{DNA}} \xrightarrow{\textit{transcription}} \underset{\text{(transcriptome)}}{\text{mRNA}} \xrightarrow{\textit{translation}} \underset{\text{(proteome)}}{\text{Protein}}$$

The monomeric units for nucleic acids are nucleotides.
Nucleotides are made up of three structural subunits

1. Sugar: ribose in RNA, 2-deoxyribose in DNA
2. Heterocyclic base
3. Phosphodiester

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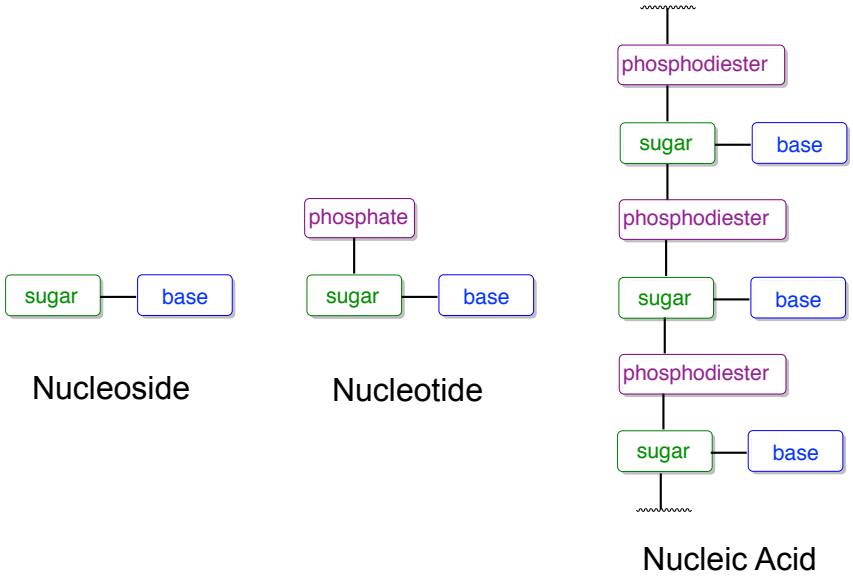
Nucleoside, nucleotides and nucleic acids



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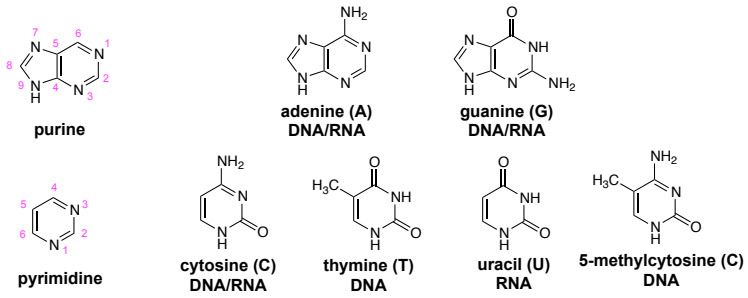
26.1: Pyrimidines and Purines. The heterocyclic bases; there are ~~five~~ *six* common bases for nucleic acids (Table 26.1, p. 1087). Note that G, T and U exist in the keto form (and not the enol form found in phenols)



26.2: Nucleosides. N-Glycosides of a purine or pyrimidine heterocyclic base and a carbohydrate. The C-N bond involves the anomeric carbon of the carbohydrate. The carbohydrates for nucleic acids are D-ribose and 2-deoxy-D-ribose

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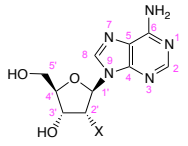
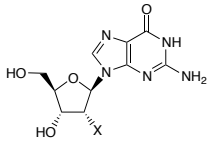
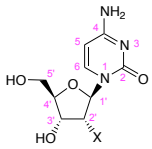
Nucleosides = carbohydrate + base (Table 28.2, p. 1089)
ribonucleosides or 2’-deoxyribonucleosides



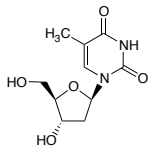
RNA: X= OH, adenosine (A)
DNA: X= H, 2'-deoxyadenosine (dA)



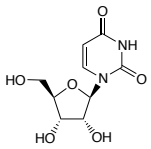
RNA: X= OH, guanosine (G)
DNA: X= H, 2'-deoxyguanosine (dG)



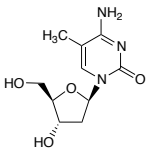
RNA: X= OH, cytidine (C)
DNA: X= H, 2'-deoxycytidine (dC)



DNA: thymidine (T)



RNA: uridine (U)



DNA: 5-methyl-2’-deoxycytidine

To differentiate the atoms of the carbohydrate from the base, the position number of the carbohydrate is followed by a ´ (prime).

The stereochemistry of the glycosidic bond found in nucleic acids is β.

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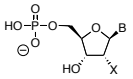
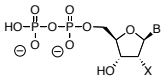
26.3: Nucleotides. Phosphoric acid esters of nucleosides.
Nucleotides = nucleoside + phosphate

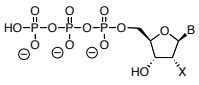
ribonucleoside (X=OH)
deoxyribonucleoside (X=H)



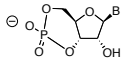
ribonucleotide 5'-monophosphate (X=OH, NMP)
deoxyribonucleotide 5'-monophosphate (X=H, dNMP)



ribonucleotide 5'-diphosphate (X=OH, NDP)
deoxyribonucleotide 5'-diphosphate (X=H, dNDP)

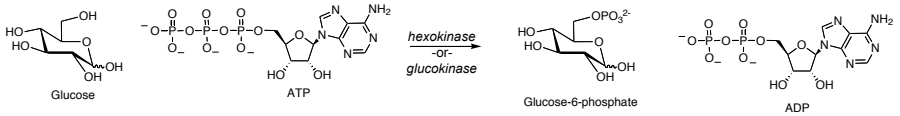


ribonucleotide 5'-triphosphate (NTP)
deoxyribonucleotide 5'-triphosphate (X=H, dNTP)



ribonucleotide
3',5'-cyclic phosphosphate (cNMP)

Kinase: enzymes that catalyze the phosphoryl transfer reaction from ATP to an acceptor substrate. M^{2+} dependent



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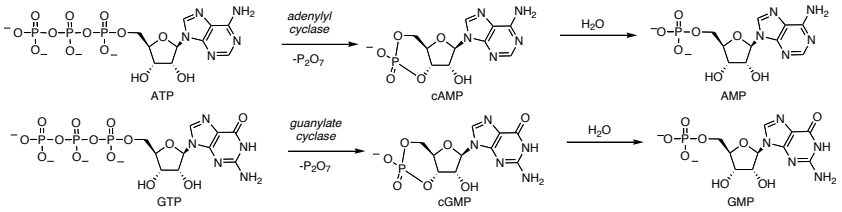
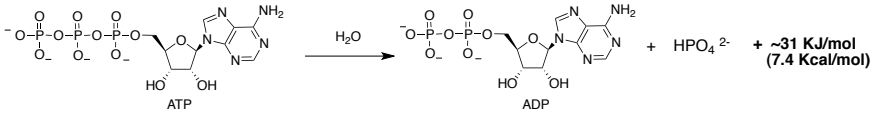


1971 Nobel Prize in Medicine or Physiology: Earl Sutherland

26.4: Bioenergetics. (Please read)

26.5: ATP and Bioenergetics. (Please read)



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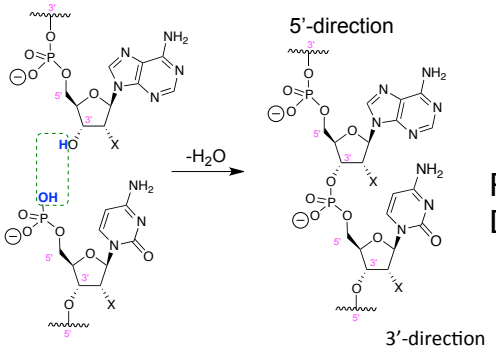
26.6: Phosphodiesters, Oligonucleotides, and Polynucleotides. The chemical linkage between nucleotide units of nucleic acids is a *phosphodiester*, which connects the 5'-hydroxyl group of one nucleotide to the 3'-hydroxyl group of the next nucleotide.



RNA, X= OH
DNA, X=H

By convention, nucleic acid sequences are written from left to right, from the 5'-end to the 3'-end.

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Nucleic acids are negatively charged

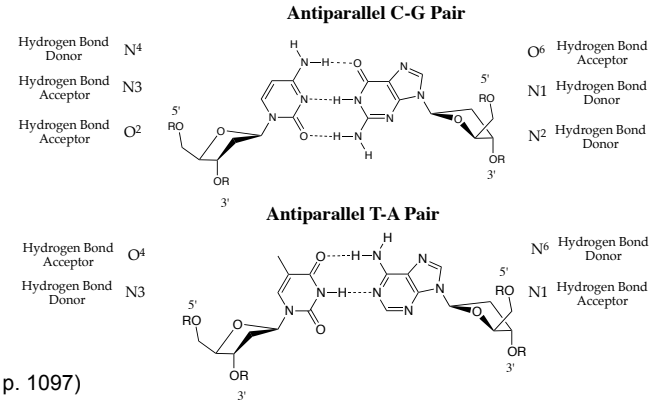
26.7: Nucleic Acids (please read).

Chargaff's Rule: A=T and C=G

26.8: Secondary Structure of DNA: The Double Helix.

Two polynucleotide strands, running in opposite directions (*anti-parallel*) and coiled around each other in a *double helix*.

The strands are held together by complementary hydrogen-bonding between specific pairs of bases.



(Fig. 26.3, p. 1097)

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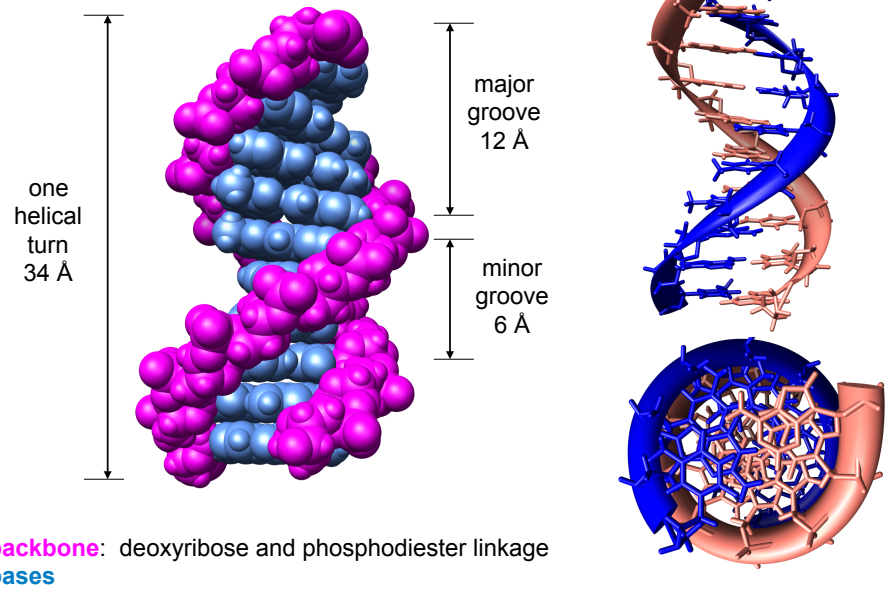
DNA double helix



backbone: deoxyribose and phosphodiester linkage

bases

(Figs. 26.4 & 26.5, p. 1098)

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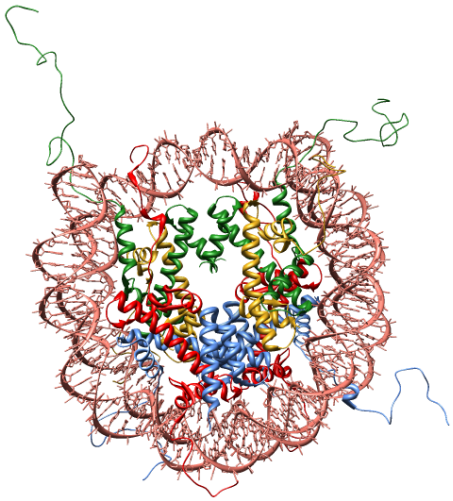
26.9: Tertiary Structure of DNA: Supercoils. Each cell contains about two meters of DNA. DNA is "packaged" by coiling around a core of proteins known as histones. The DNA-histone assembly is called a nucleosome. Histones are rich is lysine and arginine residues.



Pdb code 1kx5

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"It has not escaped our attention that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Watson & Crick

26.10: Replication of DNA.
The Central Dogma (F. Crick):

DNA $\xleftarrow{\text{replication}}$ DNA (genome) $\xrightarrow{\text{transcription}}$ mRNA (transcriptome) $\xrightarrow{\text{translation}}$ Protein (proteome)

Expression and transfer of genetic information:

Replication: process by which DNA is copied with very high fidelity.

Transcription: process by which the DNA genetic code is read and transferred to messenger RNA (mRNA). This is an intermediate step in protein expression

Translation: The process by which the genetic code is converted to a protein, the end product of gene expression.

The DNA sequence codes for the mRNA sequence, which codes for the protein sequence

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DNA is replicated by the coordinated efforts of multiple proteins and enzymes.

For replication, DNA must be unknotted, uncoiled and the double helix unwound.

Topoisomerase: Enzyme that unknots and uncoils DNA

Helicase: Protein that unwinds the DNA double helix.

DNA polymerase: Enzyme that replicates DNA using each strand as a template for the newly synthesized strand.

DNA ligase: enzyme that catalyzes the formation of the phosphodiester bond between pieces of DNA.

DNA replication is *semi-conservative*: Each new strand of DNA contains one parental (old, template) strand and one daughter (newly synthesized) strand

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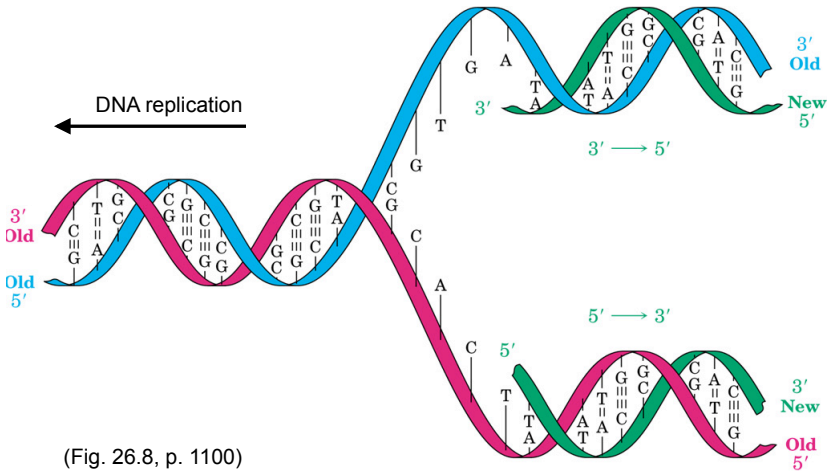
Unwinding of DNA by helicases expose the DNA bases (replication fork) so that replication can take place. Helicase hydrolyzes ATP in order to break the hydrogen bonds between DNA strands.



(Fig. 26.8, p. 1100)

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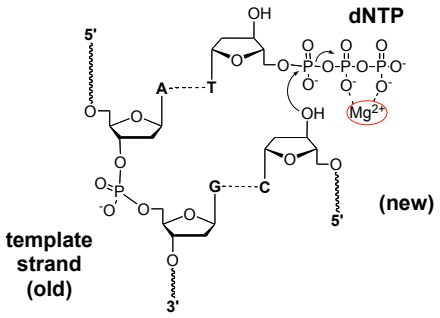
http://www.hhmi.org/biointeractive/dna-replication-advanced-detail

DNA Polymerase: the new strand is replicated from the 5'→ 3' (start from the 3'-end of the template)

DNA polymerases are Mg^{2+} ion dependent

The deoxynucleotide 5'-triphosphate (dNTP) is the reagent for nucleotide incorporation



(Fig 26.9, p. 1101)

3'-hydroxyl group of the growing DNA strand acts as a nucleophile and attacks the α-phosphorus atom of the dNTP.

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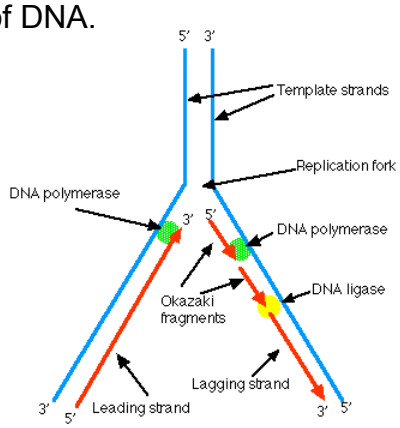
Replication of the *leading strand* occurs continuously in the 5' → 3' direction of the new strand.

Replication of the *lagging strand* occurs discontinuously. Short DNA fragments are initially synthesized and then ligated together. *DNA ligase* catalyzes the formation of the phosphodiester bond between pieces of DNA.



(Fig. 26.8, p. 1100)

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26.11 Ribonucleic Acid

RNA contains ribose rather than 2-deoxyribose and uracil rather than thymine. RNA usually exist as a single strand.

There are ~~three~~ *four* major kinds of RNA:

- messenger RNA (mRNA):
- ribosomal RNA (rRNA)
- transfer RNA (tRNA)
- microRNA (miRNA)

DNA is found in the cell nucleus and mitochondria; RNA is more disperse in the cell.

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Transcription: only one of the DNA strands is copied (coding or antisense strand). An RNA polymerase replicates the DNA sequence into a complementary sequence of *mRNA* (template or sense strand). mRNAs are transported from the nucleus to the cytoplasm, where they acts as the template for protein biosynthesis (*translation*). A three base segment of mRNA (codon) codes for an amino acid.The reading frame of the codons is defined by the start and stop codons.

THE STANDARD GENETIC CODE

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

AUG is part of the initiation signal, as well as being the codon for internal methionine.

(Table 26.4, p. 1103)

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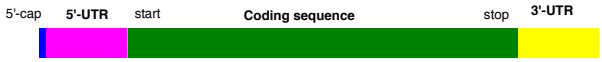

The mRNA is positioned in the ribosome through complementary pairing of the 5'-untranslated region of mRNA with a rRNA.

Transfer RNA (tRNA): t-RNAs carries an amino acid on the 3'-terminal hydroxyl (A) (aminoacyl t-RNA) and the ribosome catalyzes amide bond formation.

Ribosome: large assembly of proteins and rRNAs that catalyzes protein and peptide biosynthesis using specific, complementary, anti-parallel pairing interactions between mRNA and the anti-codon loop of specific tRNA's.

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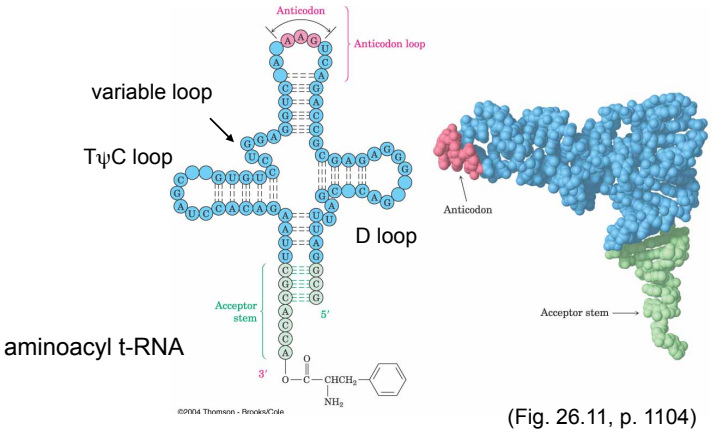
Although single-stranded, there are complementary sequences within tRNA that give it a defined conformation.

The three base codon sequence of mRNA are complementary to the "anti-codon" loops of the appropriate tRNA. The base-pairing between the mRNA and the tRNA positions the tRNAs for amino acid transfer to the growing peptide chain.



(Fig. 26.11, p. 1104)

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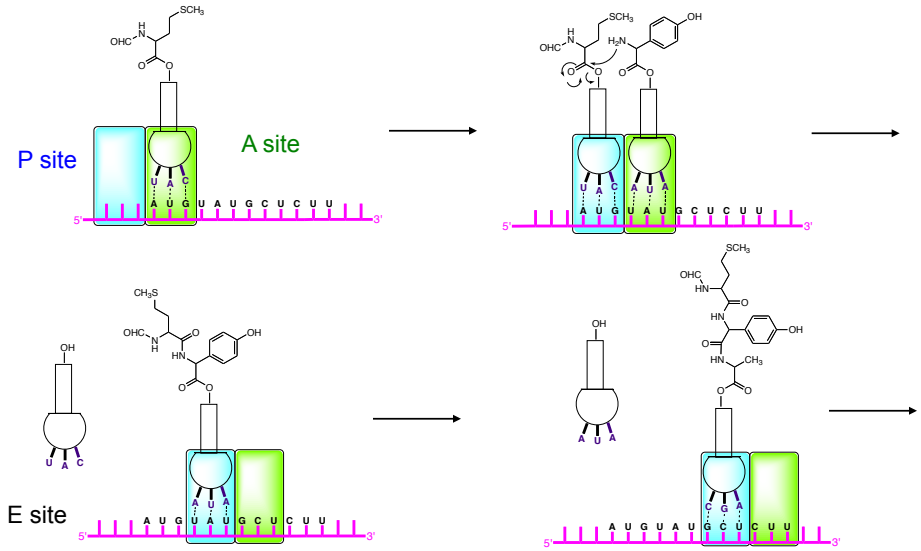
26.12: Protein Biosynthesis. Ribosomal protein synthesis



(Fig. 26.12, p. 1105)

https://www.hhmi.org/biointeractive/translation-advanced-detail

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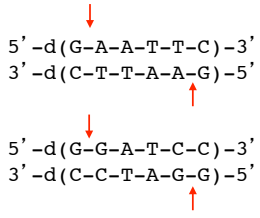
26.13: AIDS. (please read)

26.14: DNA Sequencing.

Maxam-Gilbert: relies on reagents that react with a specific DNA base that can subsequent give rise to a sequence specific cleavage of DNA

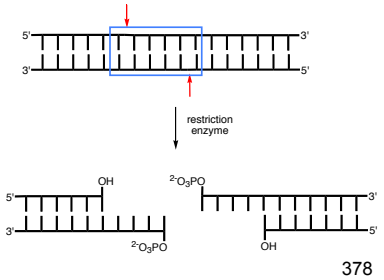
Sanger: Enzymatic replication of the DNA fragment to be sequenced with a DNA polymerase, Mg^{+2}, and dideoxynucleotides triphosphate (ddNTP) that truncates DNA replication

Restriction endonucleases: Bacterial enzymes that cleave DNA at specific sequences



EcoR I

BAM HI



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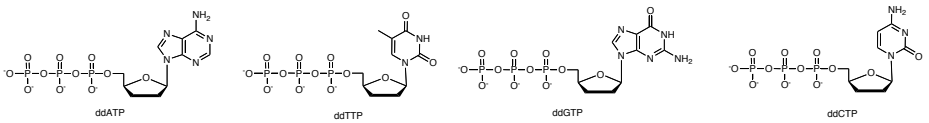
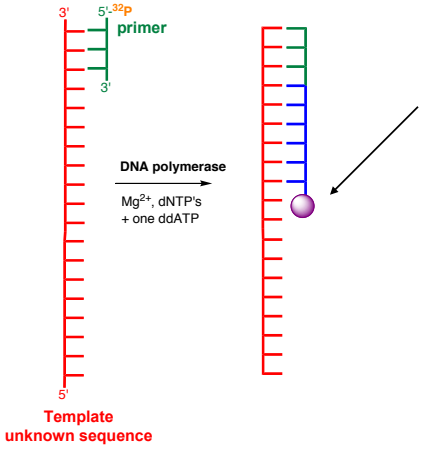
Sanger Sequencing:
key reagent: dideoxynucleotides triphosphates (ddNTP)

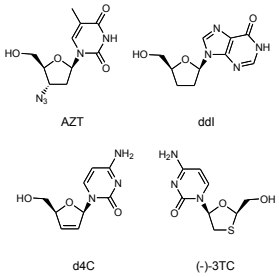




When a ddNTP is incorporated elongation of the primer is terminated

The ddNTP is specifically incorporated opposite its complementary nucleotide base

Anti-Viral Nucleosides



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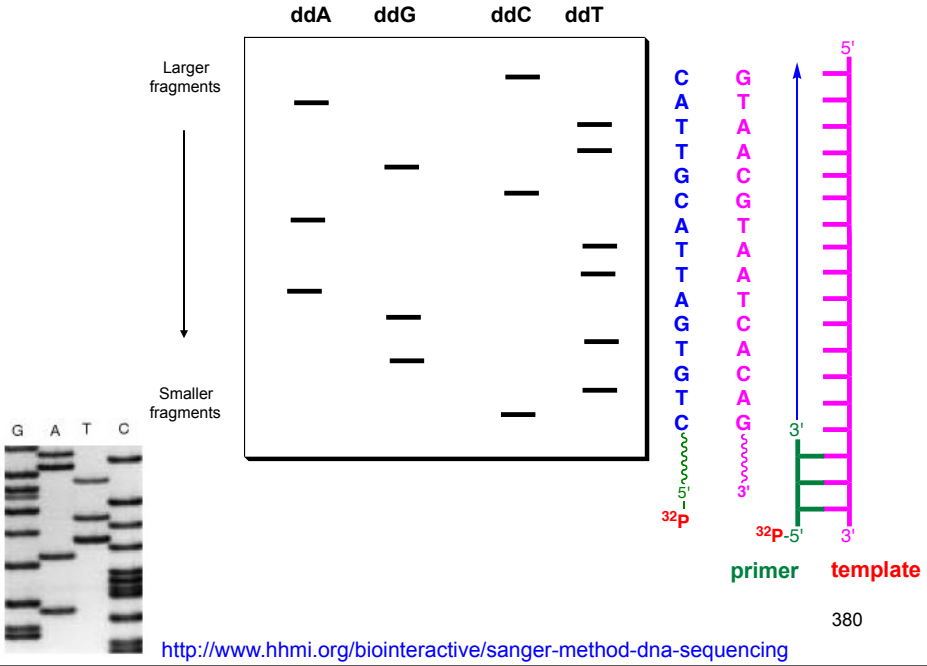
Sanger Sequencing



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http://www.hhmi.org/biointeractive/sanger-method-dna-sequencing

26.15: The Human Genome Project. (please read)
26.16: DNA Profiling and Polymerase Chain Reaction (PCR).
method for amplifying DNA using a DNA polymerase, dNTPs and cycling the temperature.

Heat stable DNA Polymerases (from *archaea*):

Taq: thermophilic bacteria (hot springs)- no proof reading
Pfu: geothermic vent bacteria- proof reading

Mg^{2+}
two Primer DNA strands (synthetic, large excess)
one sense primer and one antisense primer
one Template DNA strand (double strand)
dNTP's

1 x 2 = 2 x 2 = 4 x 2 = 8 x 2 = 16 x 2 = 32 x 2 = 64 x 2 = 128 x 2 = 256 x 2 = 512 x 2 = 1,024 x 2 = 2,048 x 2 = 4,096 x 2 = 8,192 x 2 = 16,384 x 2 = 32,768 x 2 = 65,536 x 2 = 131,072 x 2 = 262,144 x 2 = 524,288 x 2 = 1,048,576

In principle, over one million copies per original, can be obtained after just twenty cycles

KARY B. MULLIS, 1993 Nobel Prize in Chemistry for his invention of the polymerase chain reaction (PCR) method.

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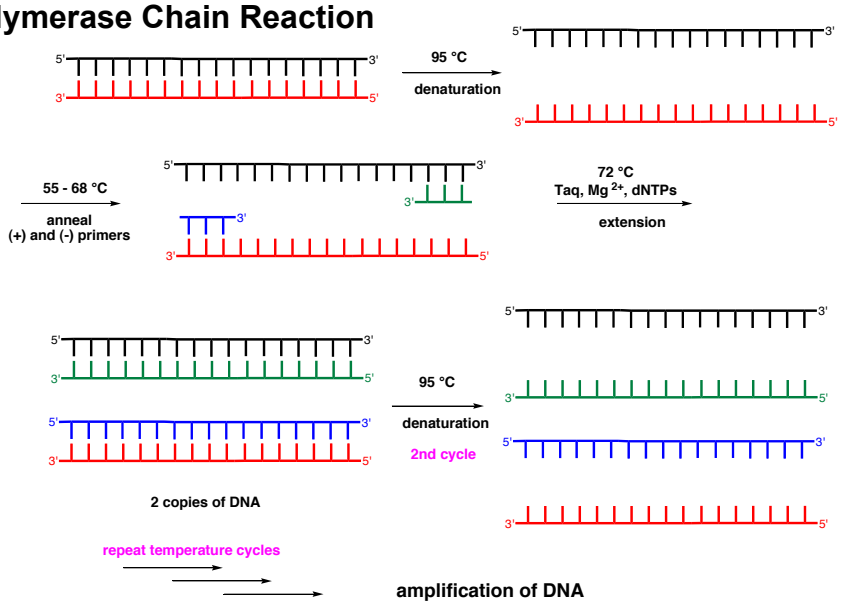
Polymerase Chain Reaction



(Fig. 26.14, p. 1109-10)

For a PCR animation go to: http://www.youtube.com/watch?v=ZmqqRPISg0g

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