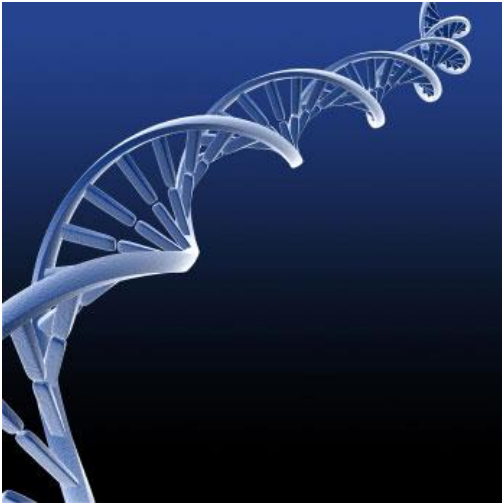


Chapter 4

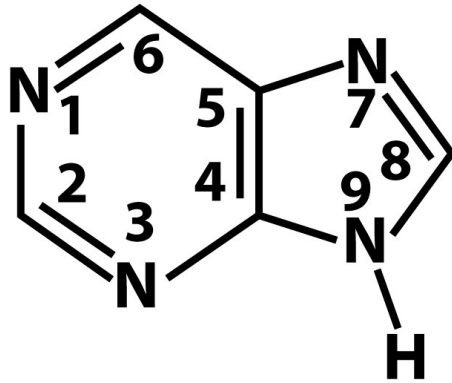


- Today objectives
 - Be familiar with the structure of bases, nucleosides and nucleotides
 - Understand forces which stabilize DNA/RNA structures

Small quiz



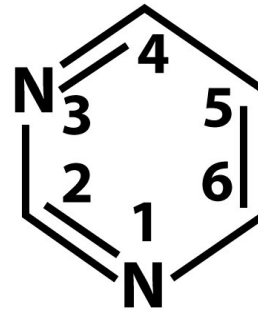
Bases:



Purine

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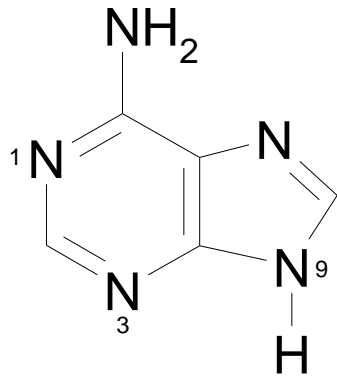
Adenine and Guanine



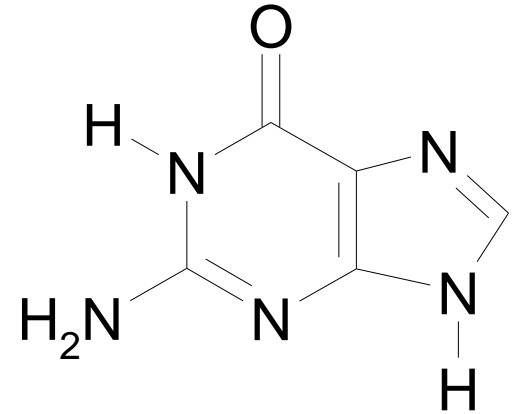
Pyrimidine

Thymine, Cytosine and Uracyl

Purine bases

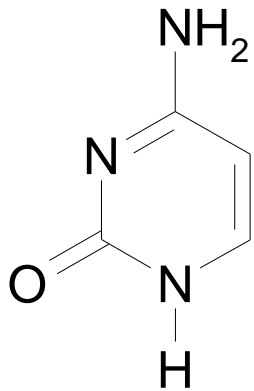


Adenine (6-aminopurine)

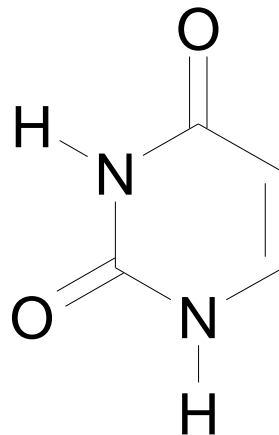


Guanine (2-amino-6-oxo purine)

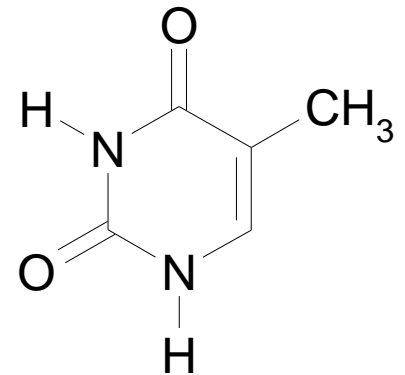
Pyrimidine bases



Cytosine
(2-oxy-4-amino pyrimidine)



Uracil (2-oxy-4-oxy-pyrimidine)

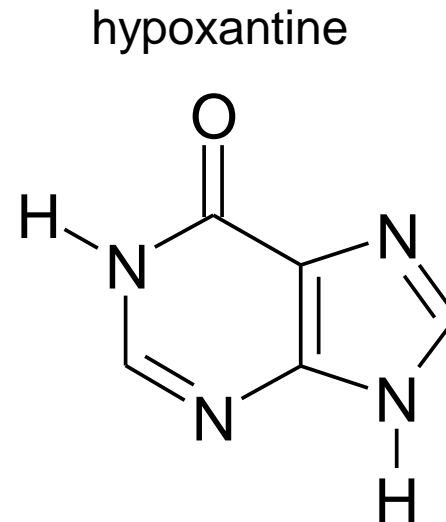
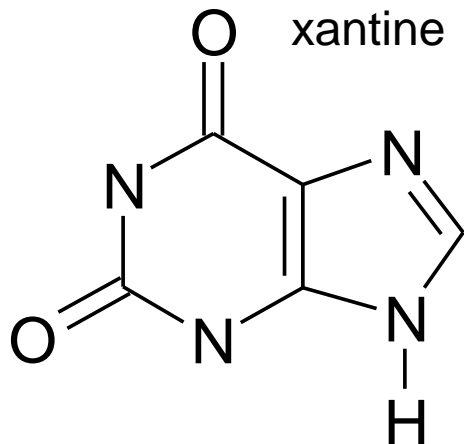


Thymine
(2-oxy-4-oxy-5-methyl pyrimidine)

Minor bases found in nucleic acids:

- methylated bases
- in RNA,
- in small amount
- methylation occurs after RNA syntheses

2-methyladenine; 1-methylguanine, 5-methylcytosine, and 5-hydroxymethylcytosine

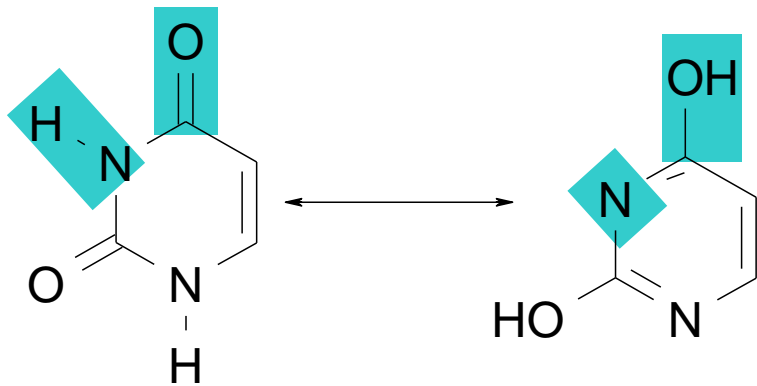


May occur in DNA, product of oxidative deamination of DNA

Properties of pyridines and purines

All bases exist in tautomeric forms

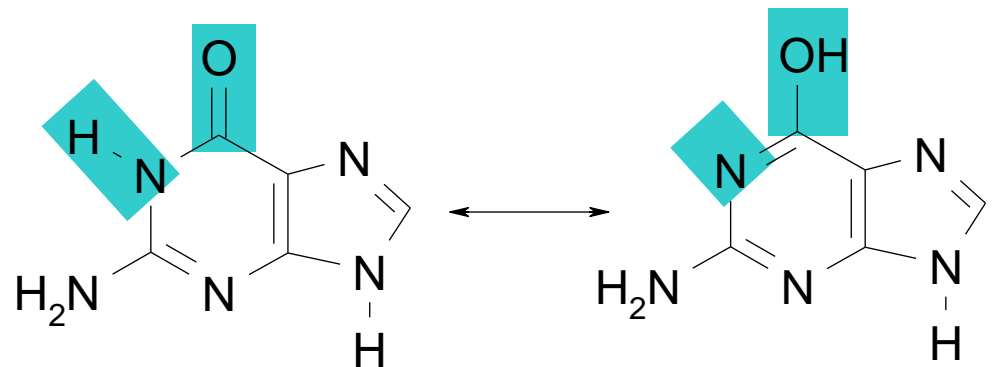
a) uracyl



keto or lactam

enol form or lactim

b) guanine



keto form

enol form

5'-CMP pKa = 4.3 (N-3)

5' - UMP pKa = 9.5 (N-3)

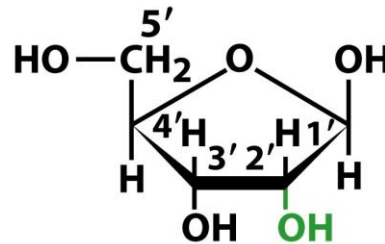
5' -AMP pKa = 3.8 (N-1)

5'-GMP pKa = 9.4 (N-1)

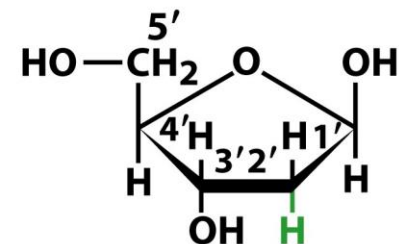
5' -GMP pKa = 9.5 (N-3)

Nucleosides – glycosylated bases

-base covalently bound via a N-glycosidic bond to an aldopentose



Ribose



Deoxyribose

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RNA:

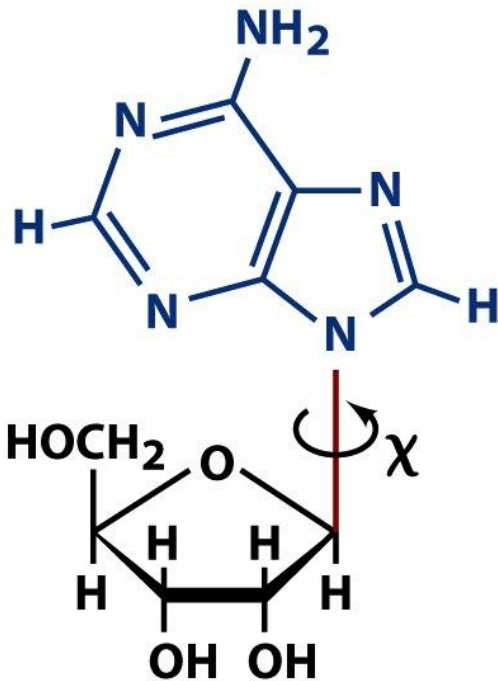
adenosine
guanosine
cytidine
uridine

DNA:

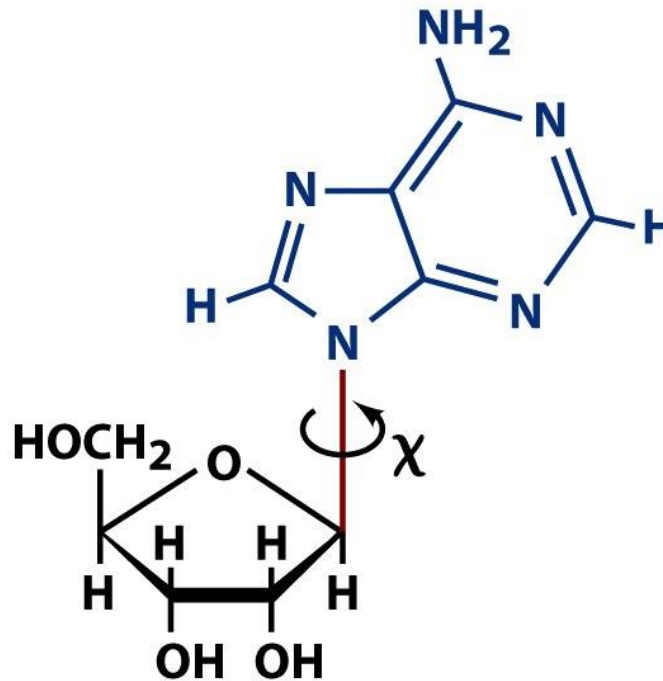
deoxyadenosine
deoxyguanosine
deoxycytidine
deoxythymidine

Conformation of β N-glycosidic bond

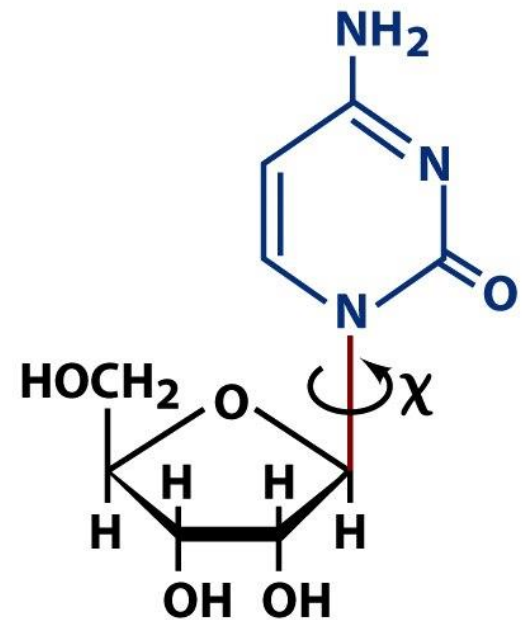
Purin bases *syn* conformation – bicyclic purine is over the sugar
anti conformation – H8 is over the sugar
pyrimidine only in *anti* conformation
(O₂ represents a steric hindrance)



***syn*-Adenosine**



***anti*-Adenosine**



***anti*-Cytidine**

Conformation of pentose

Pentose sugar is not planar

Atom in position C'2 or C'3 is out of plane – sugar puckering

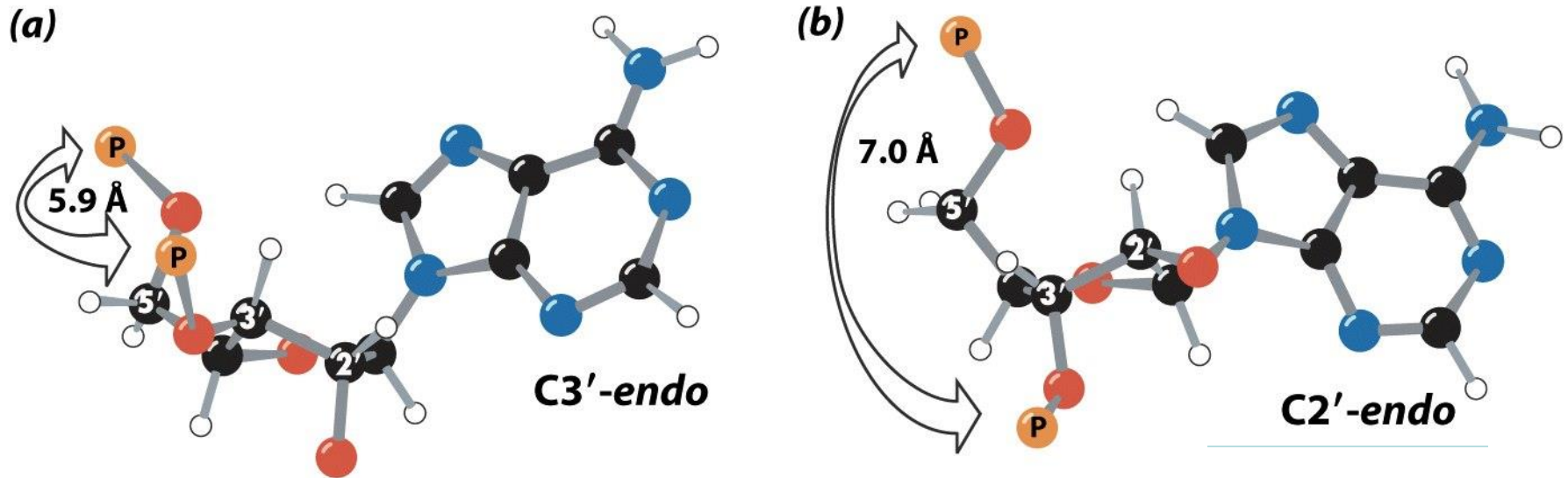


Figure 23-7 Fundamentals of Biochemistry, 2/e
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(north conformation)

(south conformation)

Nucleotides

- nucleoside having a phosphoryl group linked to a hydroxyl group on the pentose

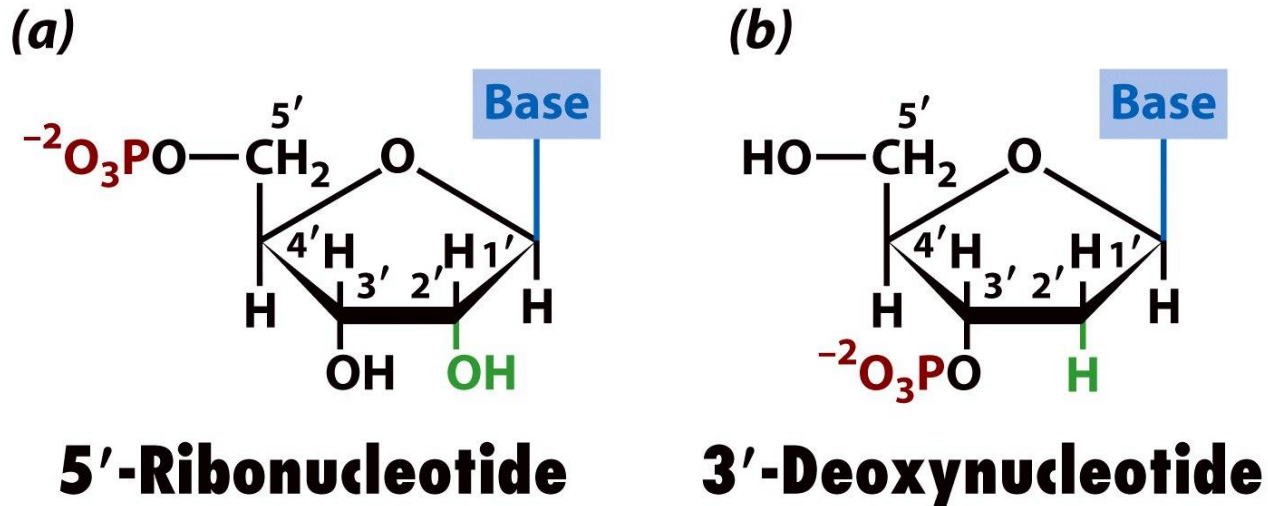
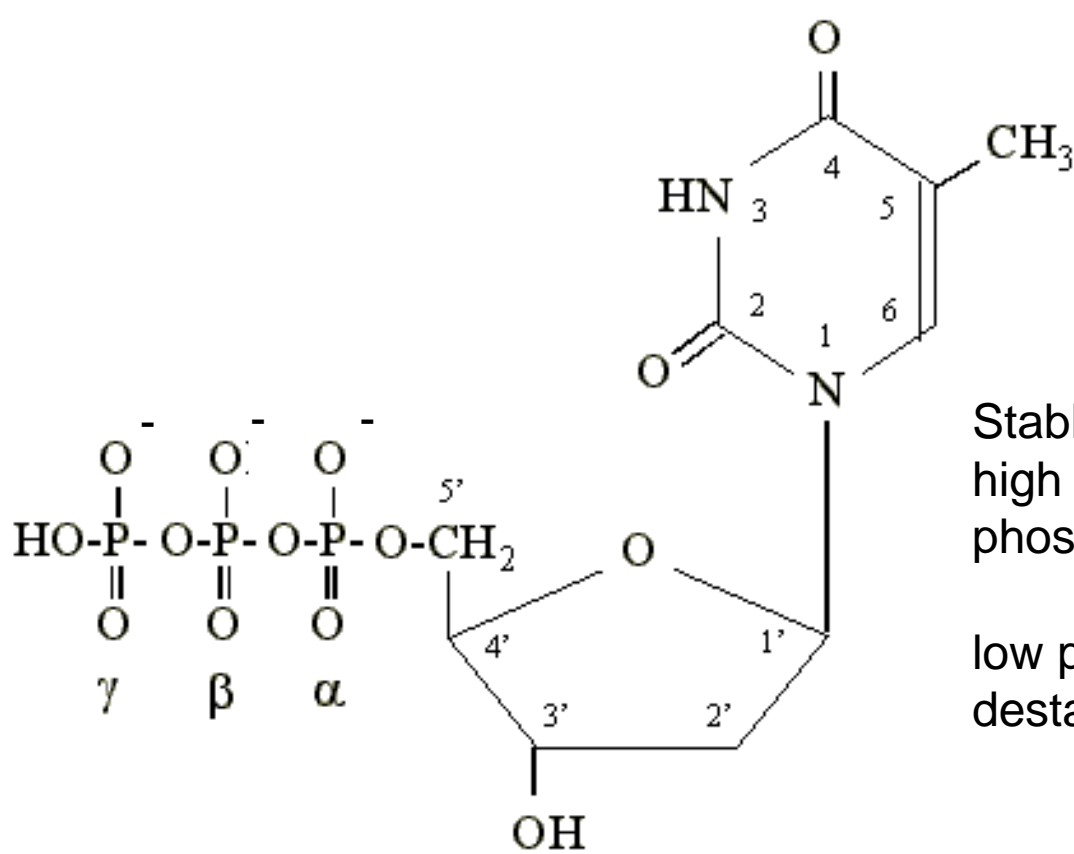


Figure 3-1 Fundamentals of Biochemistry, 2/e
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- phosphate group usually in the position C5' – nucleoside-5'-phosphate
- phosphate group: monophosphate, diphosphate or triphosphate
- nucleotides are anionic – in the cell associated with Mg^{2+}



Stable over wide pH range
 high pH – hydrolyses of
 phosphoester bond

low pH – glycosidic bond is
 destabilised

**2'-deoxy Thymidine triphosphate
 (nucleotide)**

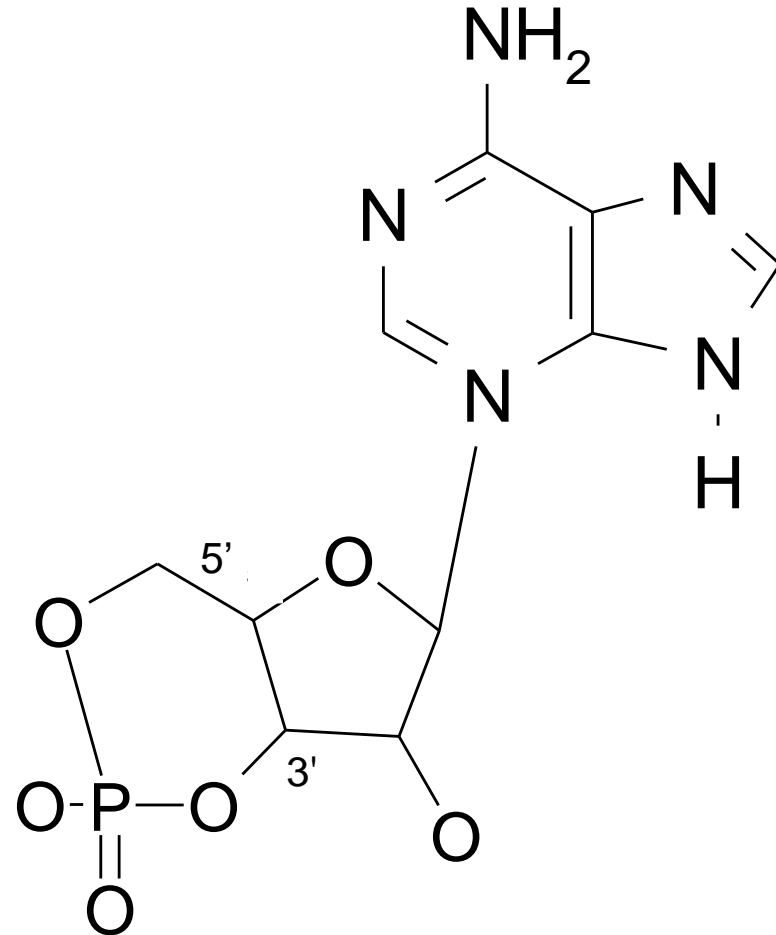
- phosphoryl group: α , β , and γ
- Nucleotides (nucleosides and bases) are soluble in water
- first pKa of phosphate group ~ 1
- Second pKa of phosphate group 6.5

Nucleotides functions

- A) polynucleotides
- Building block of nucleic acids
ribose and deoxy ribose
- B) mononucleotides
majority have ribose,
phosphate group is attached to C5'
- Regulators of cellular metabolism
(cAMP, cGMP) – ribose as a sugar
- Prime source of chemical energy
- ATP – for majority of metabolic
processes
- GTP – in protein synthesis

base and sugar part do not transmit
energy

Base part seems to be a signaling part

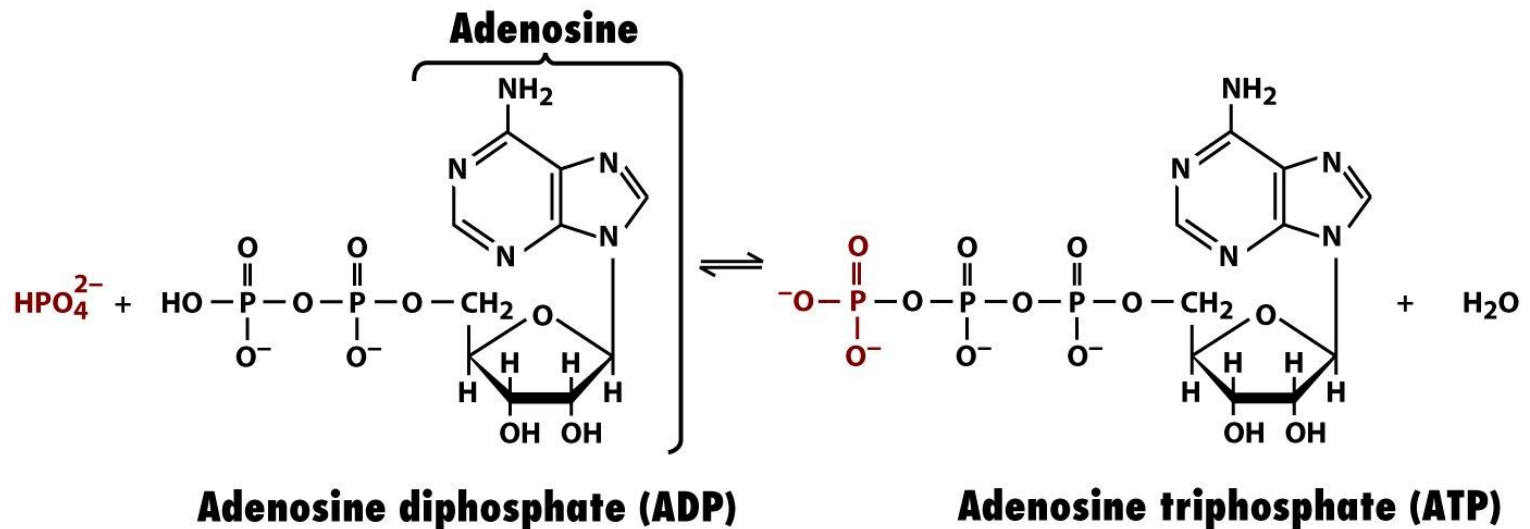


3',5' cyclic AMP

ATP – energy carrier or storage molecule

ATP synthesized from ADP during photosynthesis and in respiratory chain

Concentration of cellular ATP ~ 5 mM

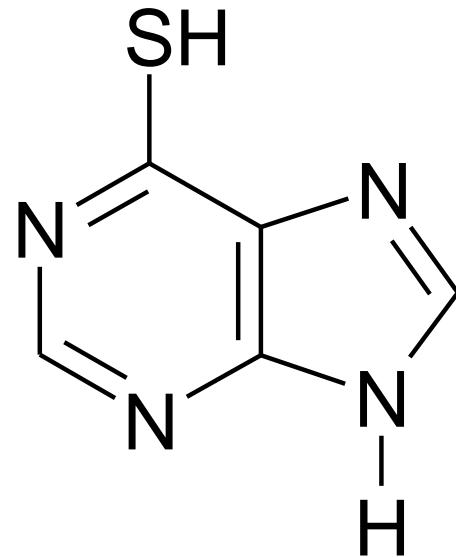
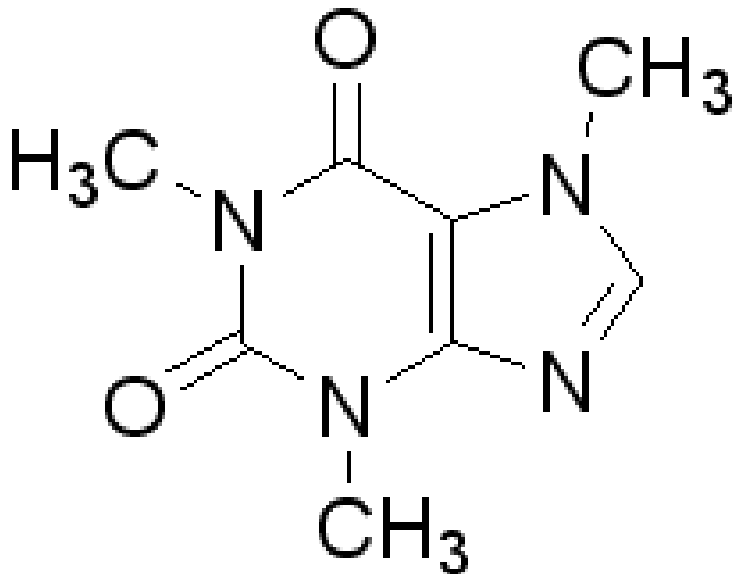


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Purines and pyrimidines with physiological activity

Caffeine: trimethyl derivate of 2,6,-oxy-purine
stimulant effect
block interaction of adenosine with neuronal receptors

6-mercaptapurine: block syntheses of nucleic acids
use for leukemia treatment by blocking
uncontrolled cell division

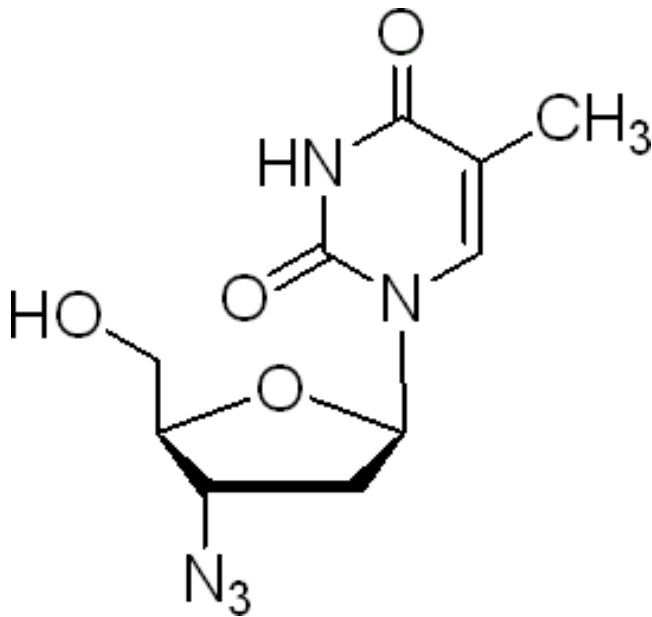


Nucleosides with physiological activity

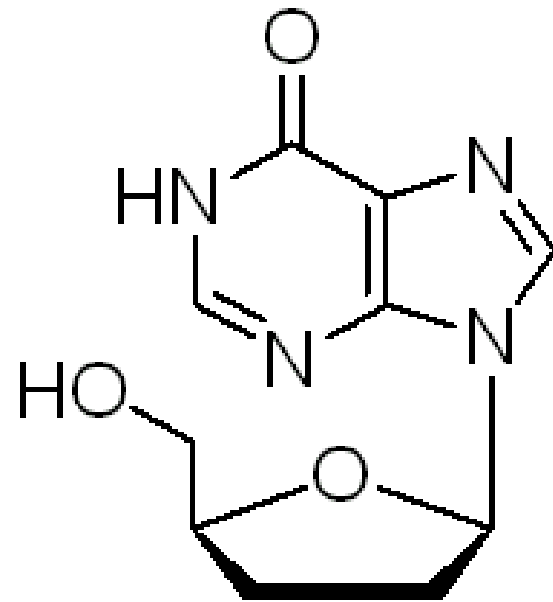
Treatment of HIV

3'-azidodeoxythymidine

2',3'-dideoxyinosine



Inhibitor of reverse transcriptase



Antiviral activity

Nucleic Acids – single strain

- ✓ polymers of nucleotides or deoxynucleotides
- ✓ nucleotides are linked via phosphodiester bond; phosphate bridge between 5' and 3' position on two ribose units
- ✓ convention: sequence of bases is written from 5' end (left) to 3' end (right)
 - 5' end C5' end is free
 - 3' end C3' end is free

-sugar –phospho-diester bond is uniform, diversity arises from bases

Single strain and double strain DNA

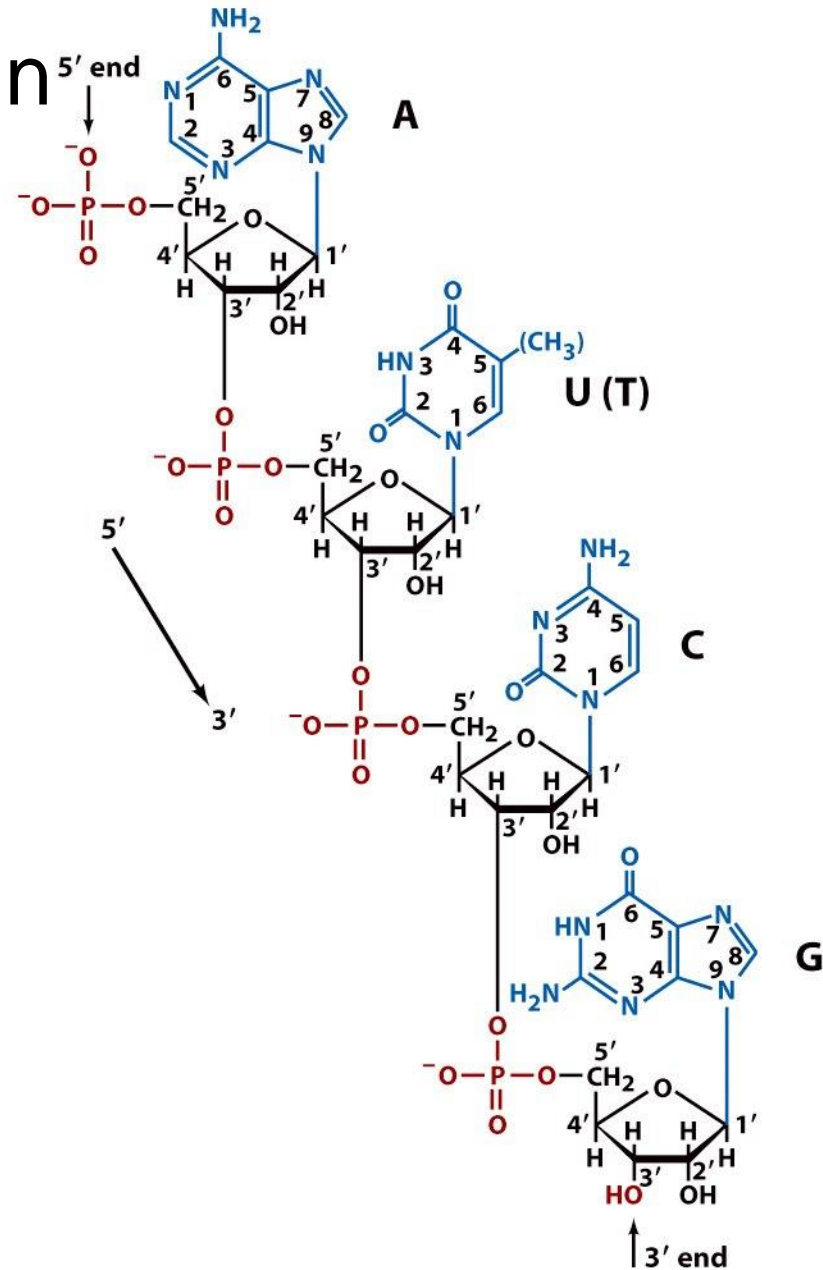


Figure 3-3a Fundamentals of Biochemistry, 2/e
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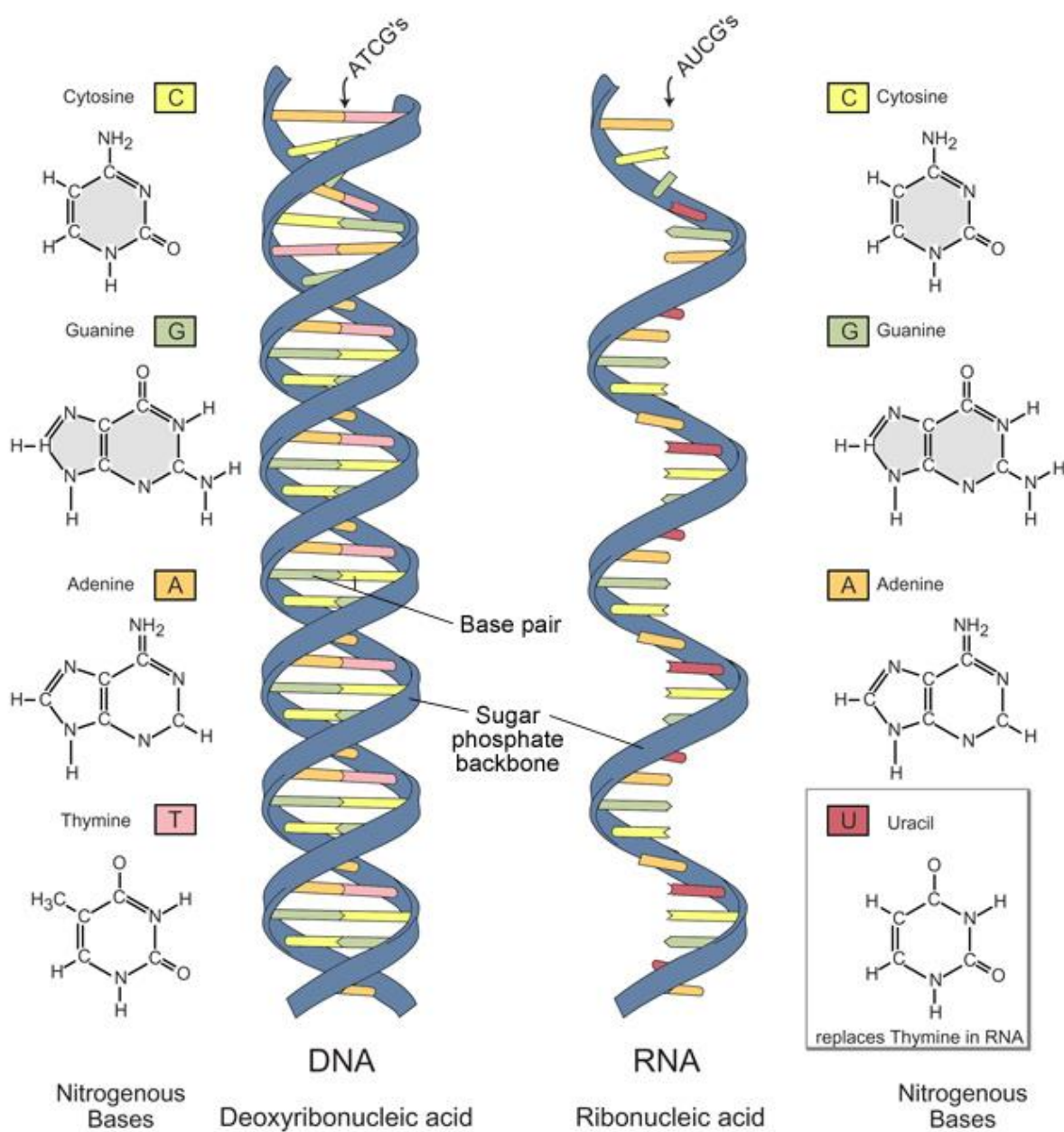


Image adapted from: National Human Genome Research Institute.

Schematic presentation

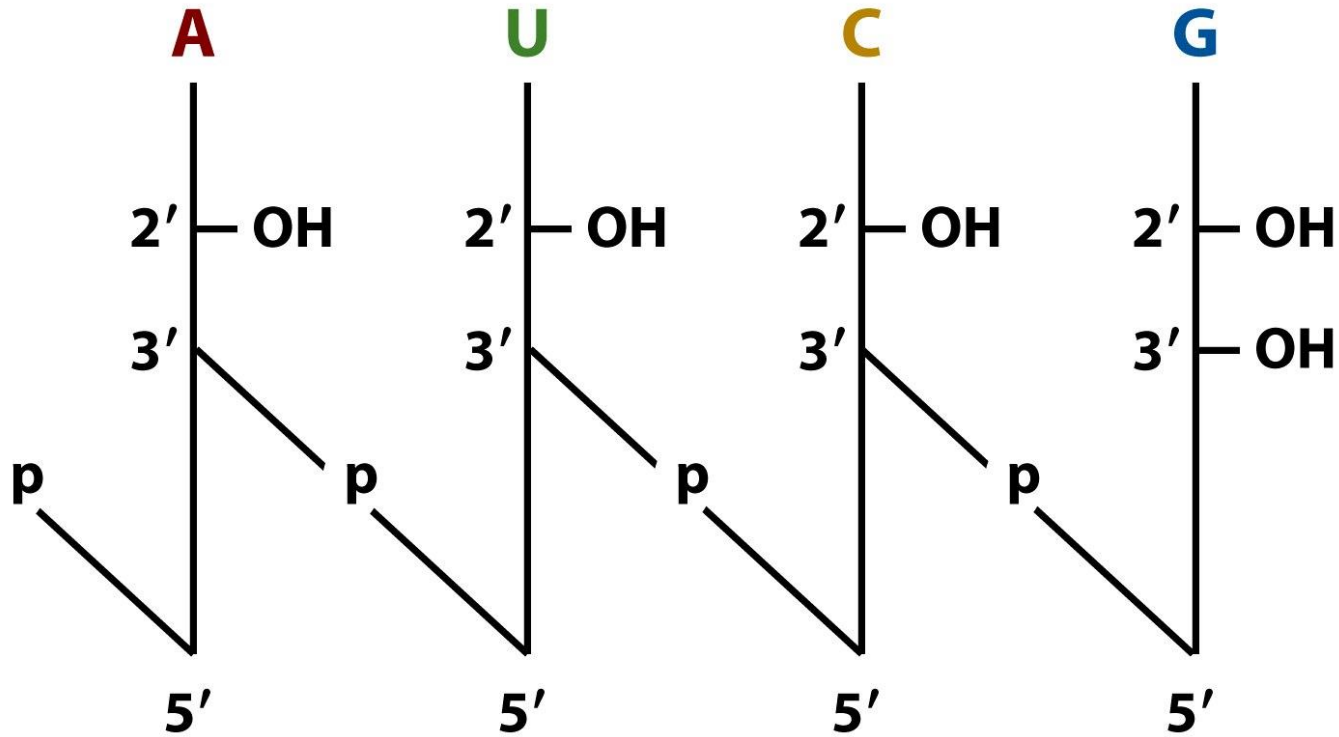


Figure 3-3b Fundamentals of Biochemistry, 2/e
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pApUpCpG or pAUCG

in case of deoxytetranucleotide: d(pApTpCpG) or d(pATCG)

The sequence is written in 5' to 3' direction, complementary sequence

DNA

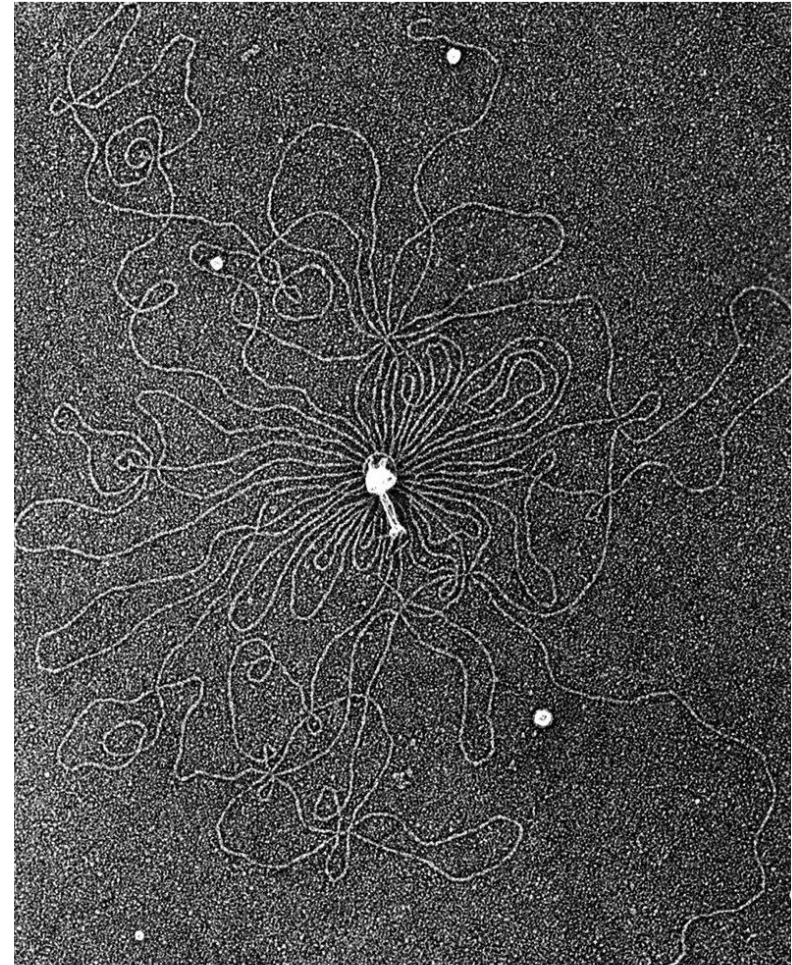
Occurs in prokaryote cells and in eukaryote cells in nucleus, also in mitochondria and in chloroplast

Circular DNA-bacteria and viruses
Linear DNA – Eukaryotes and adenovirus

Size and composition depends on the species of origin

E. coli – 9×10^6 nucleotides; 4.65×10^6 bp,
1400 μm length

Human DNA – 3×10^9 bp, $1\text{-}2 \times 10^6$ μm
length



Chapter 23 Opener Fundamentals of Biochemistry, 2/e

DNA composition

1951 Erwin. Chargaff:

isolated DNA from various species and determined base composition

Regardless species, adenine : thymine 1:1

cytosine : guanine 1:1

Chargaffs rules: **[A] = [T]**
[C] = [G]
[purine] = [pyrimidines]

DNA base composition varies between organisms: 25% to 75% of C+G in different species of bacteria

In related species amount is similar; 39% to 46% of G+C in mammals

For a human DNA, Chaffards results yielded following molar ratios:

$$A/G = 1.56,$$

$$T/C = 1.75$$

$$A/T = 1$$

$$G/C = 1$$

1950's R.E. Franklin

X-ray diffraction studies of DNA, two or more chains must coil around each other to form a helix.

1953 J.D. Watson and F.H.C. Crick – identified hydrogen bonding and proposed double helix structure

- ✓ two polynucleotide chains wind around a common axes
- ✓ two chains run in opposite direction, are antiparallel;
- ✓ sugar phosphate backbones run on the periphery
- ✓ Bases are located in the middle
- ✓ Minor and major groove located on the surface, exposure of the edges of bp
- ✓ A base is hydrogen bound to a complementary base from the opposite chain

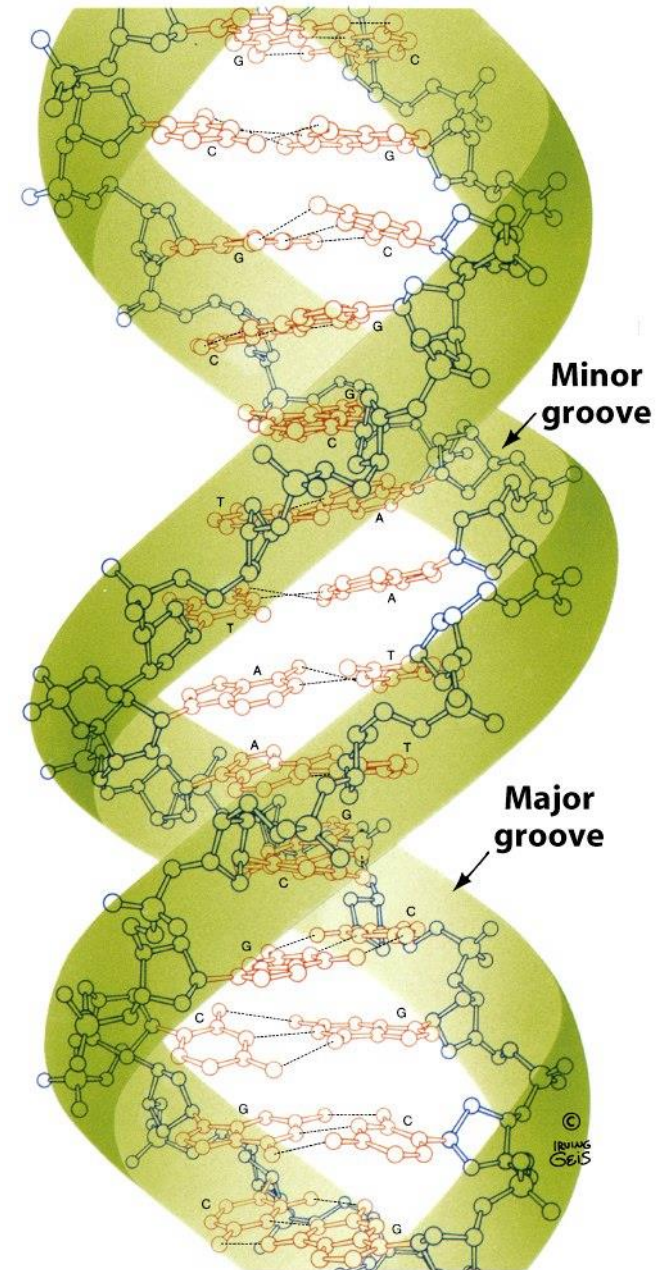
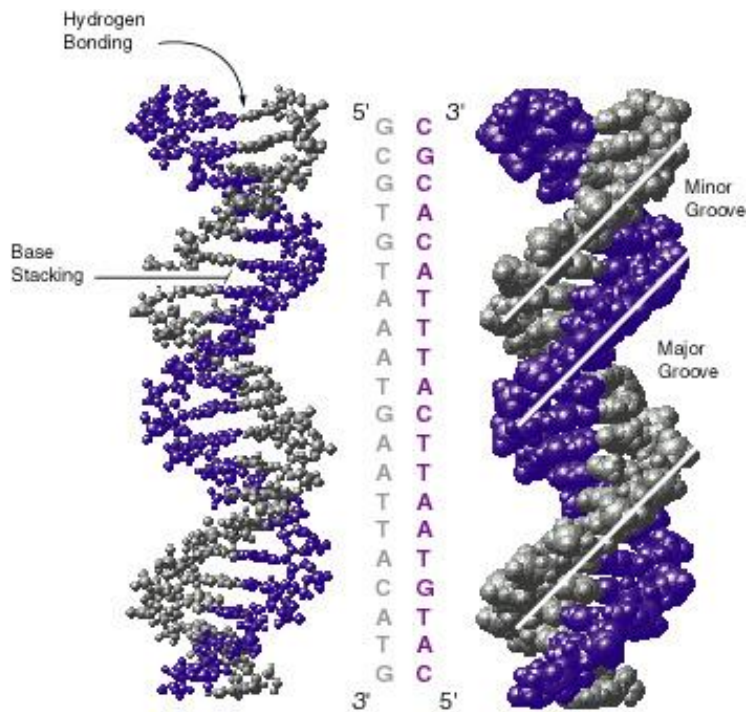


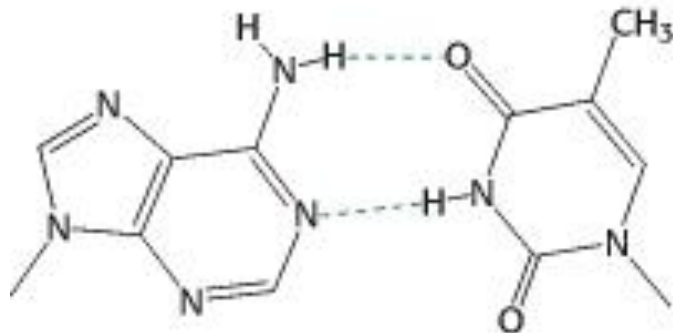
Figure 3-6 Fundamentals of Biochemistry, 2/e



B conformation

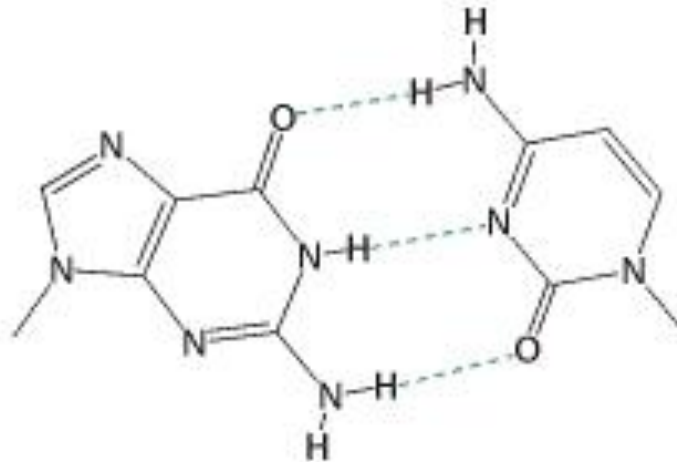
- most common
- right handed
- 20 Å diameter
- 10.5 bp per turn
- helical pitch 34Å

Miesfeld / Applied Molecular Genetics
Fig. 01.03. DNA Structure.



Adenine (A)

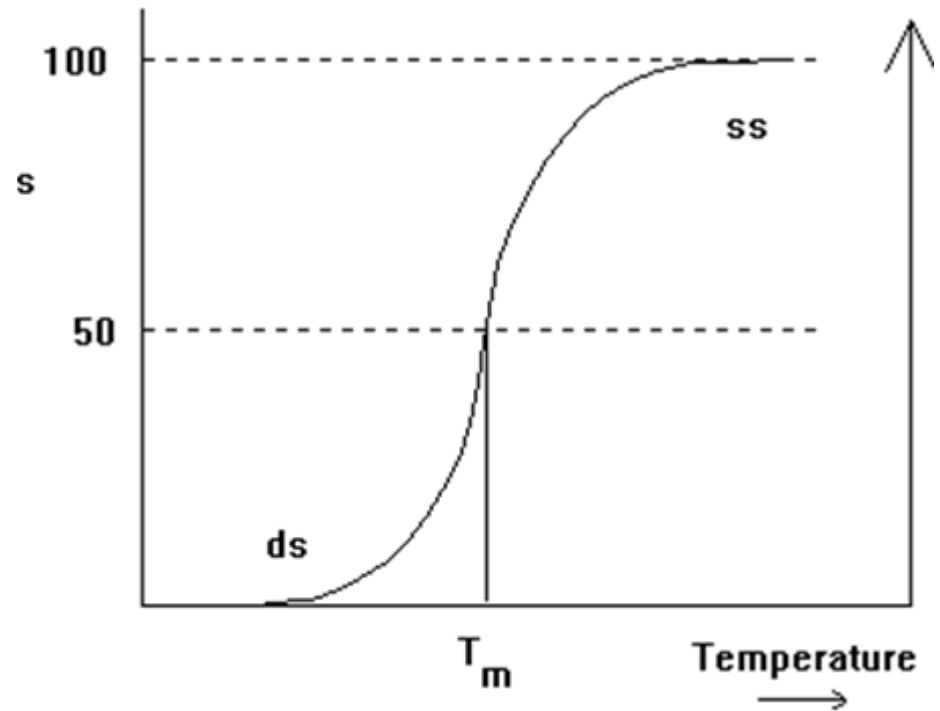
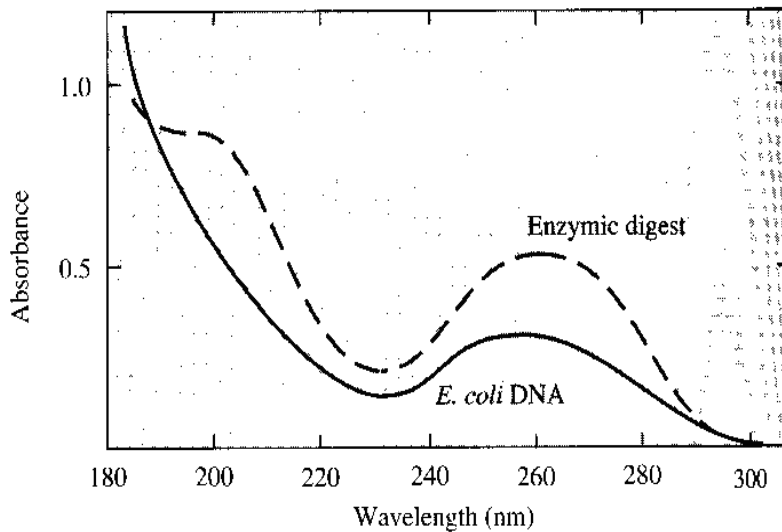
Thymine (T)



Guanine (G)

Cytosine (C)

DNA absorption spectra and denaturation



Melting temperature is proportional to GC content

DNA synthesis

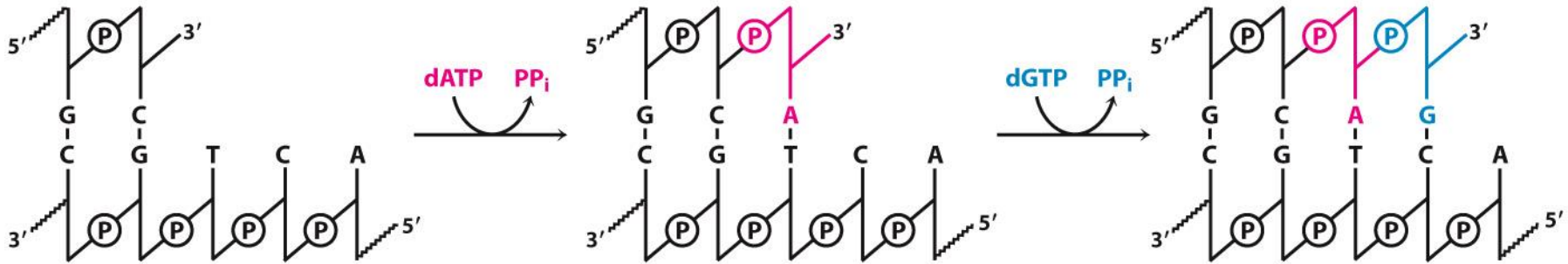


Figure 4.24
Biochemistry, Eighth Edition
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Requires:

nucleotides

primer (new DNA strand complementary to parent DNA)

DNA polymerase (coming nucleotides are attached to free 3' OH group)

synthesis is rppfreaded by DNA polymerase

- DNA replication (prokaryotes and eukaryotes):
complex process ~ 20 proteins
catalyzed by DNA polymerase



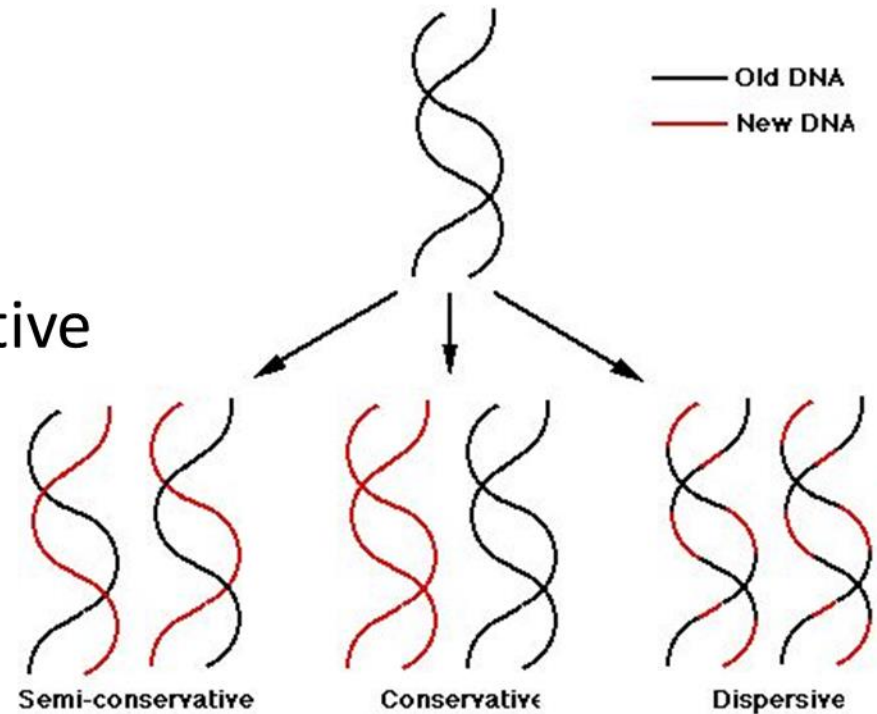
nucleotides

DNA template

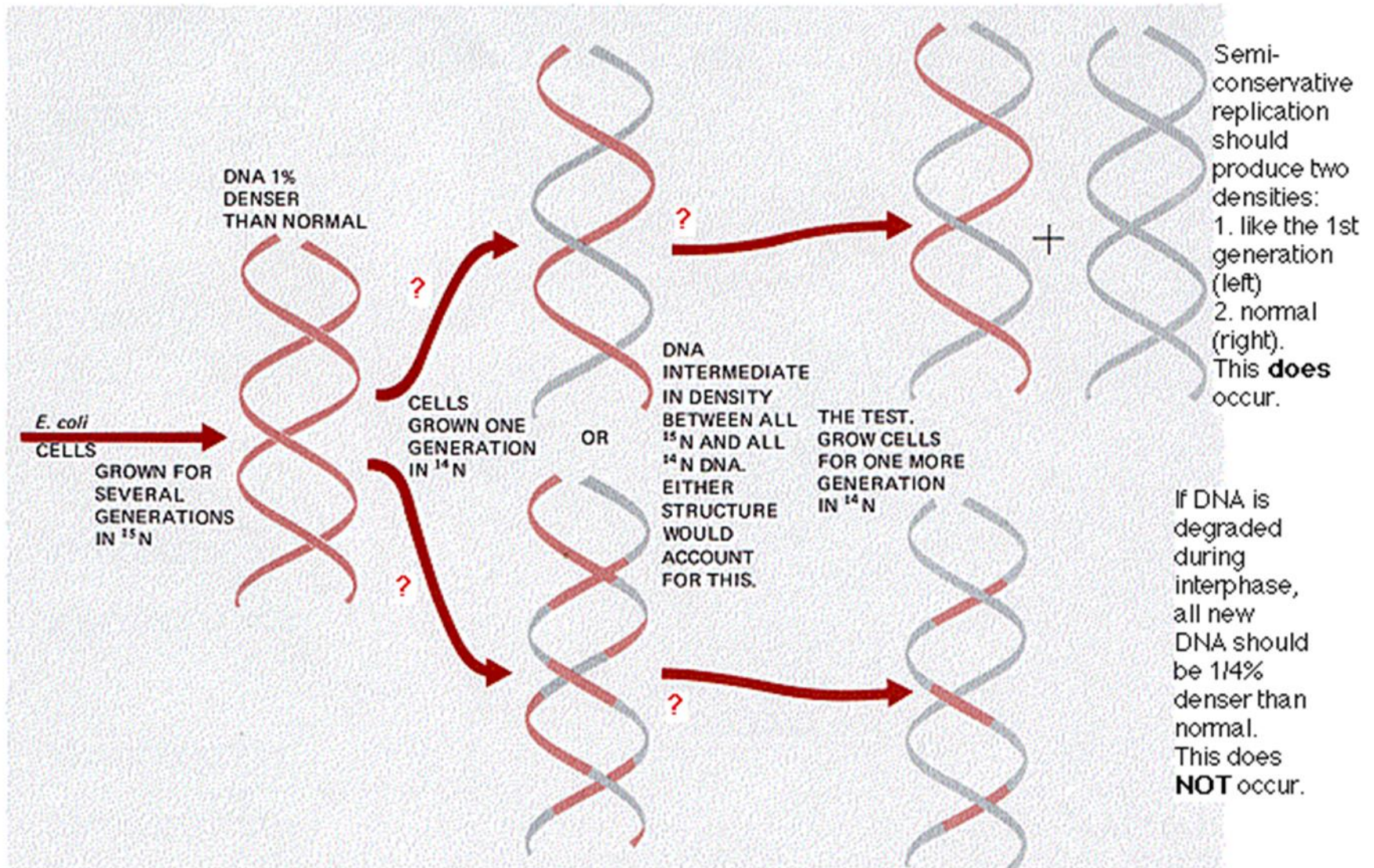
Primer (free 3' OH group)

High fidelity reaction

DNA synthesis is semiconservative



M. S. Meselson and F. W. Stahl. experiment



- **RNA molecules**

- mRNA: template for protein synthesis
- t-RNA carries AA residues in a sequence order of mRNA (codon complementarity)
- rRNA – ribosomes, catalytic site for protein synthesis
- **Small nuclear RNA** - participate in exon splicing
- **microRNA** – complementary to mRNA and inhibit protein synthesis
- Small interfering RNA (binds to mRNA and facilitate its degradation)
- RNA- part of telomerase (maintain the length of chromosome)
- RNA molecules part of signal-recognition particles involved in intracellular protein distribution

RNA synthesis

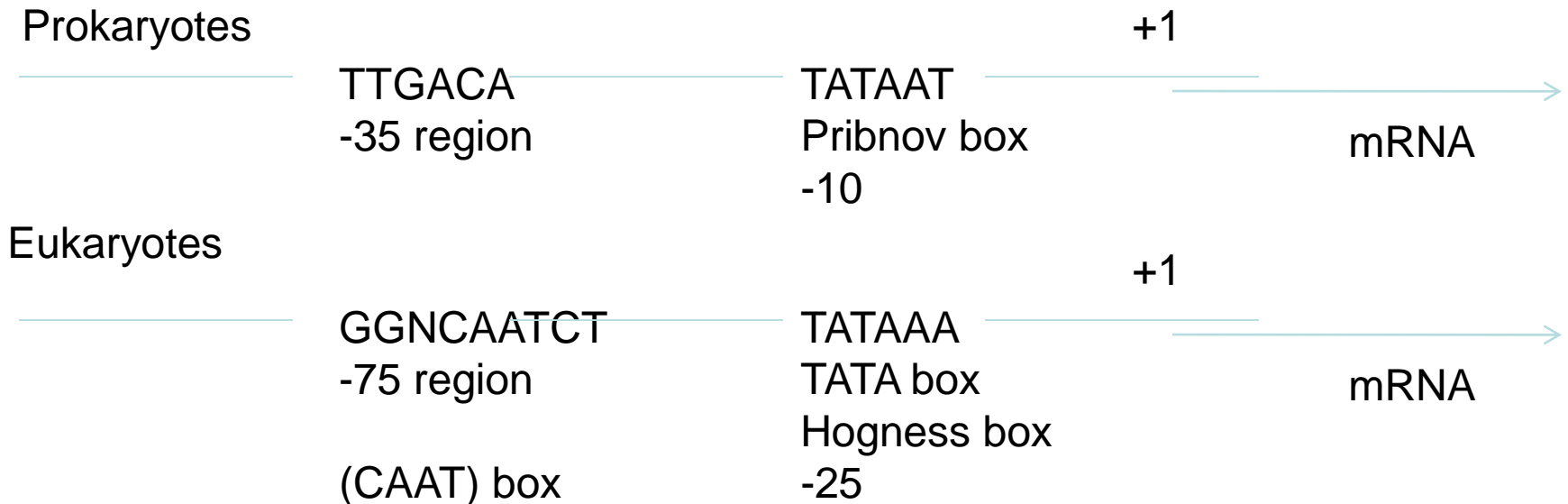
- $(\text{RNA})_n + \text{dNTP} \leftrightarrow (\text{RNA})_{n+1}$
- DNA template
- Nucleotides triphosphates (ATP, GTP, UTP, CTP)
- Divalent metals
- **RNA polymerase**
- No need of primer
- 5' -> 3' direction
- no proofreading

5'- **GCGGCGACGC**
3' -CGCCGCTGCG
5'-**GCGGCGACGC**

mRNA
template
coding strand

Promoters

- Promotor sites are recognize by RNA polymerase and indicate the position of first nucleotide to be transcribed



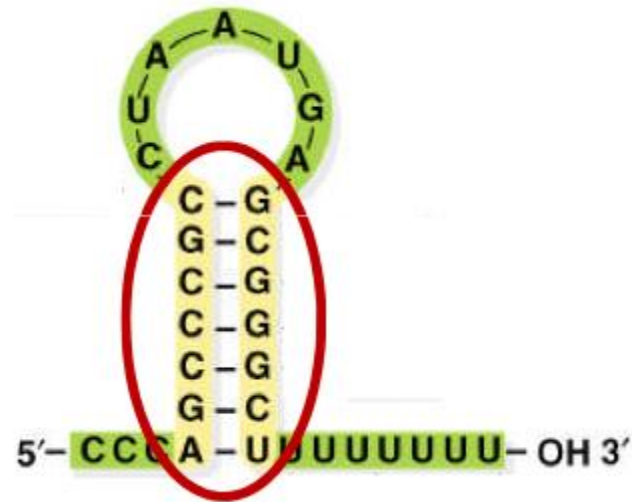
- Termination of RNA synthesis:

- In bacteria:

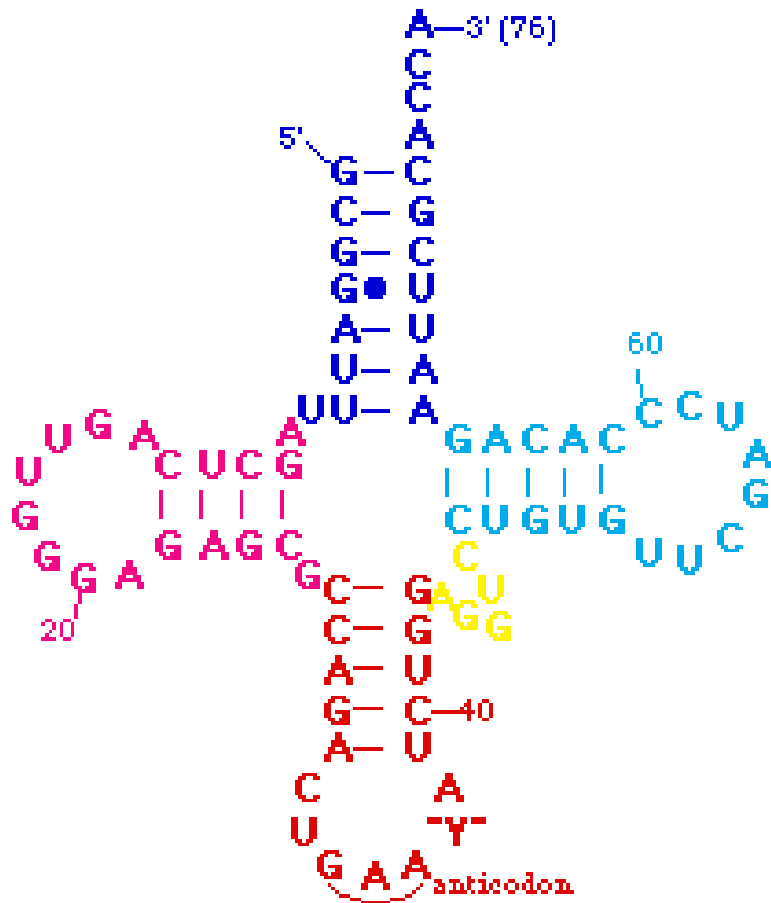
- Rho protein- facilitates RNA polymerase dissociation

- Base pair hairpin:

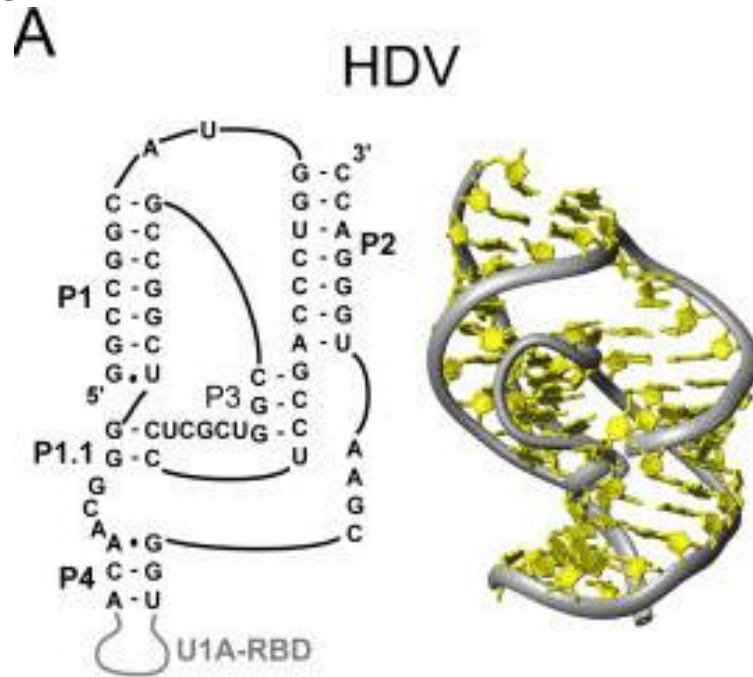
RNA polymerase dissociate from the DNA when hairpin is formed on synthesized RNA



t-RNA structure

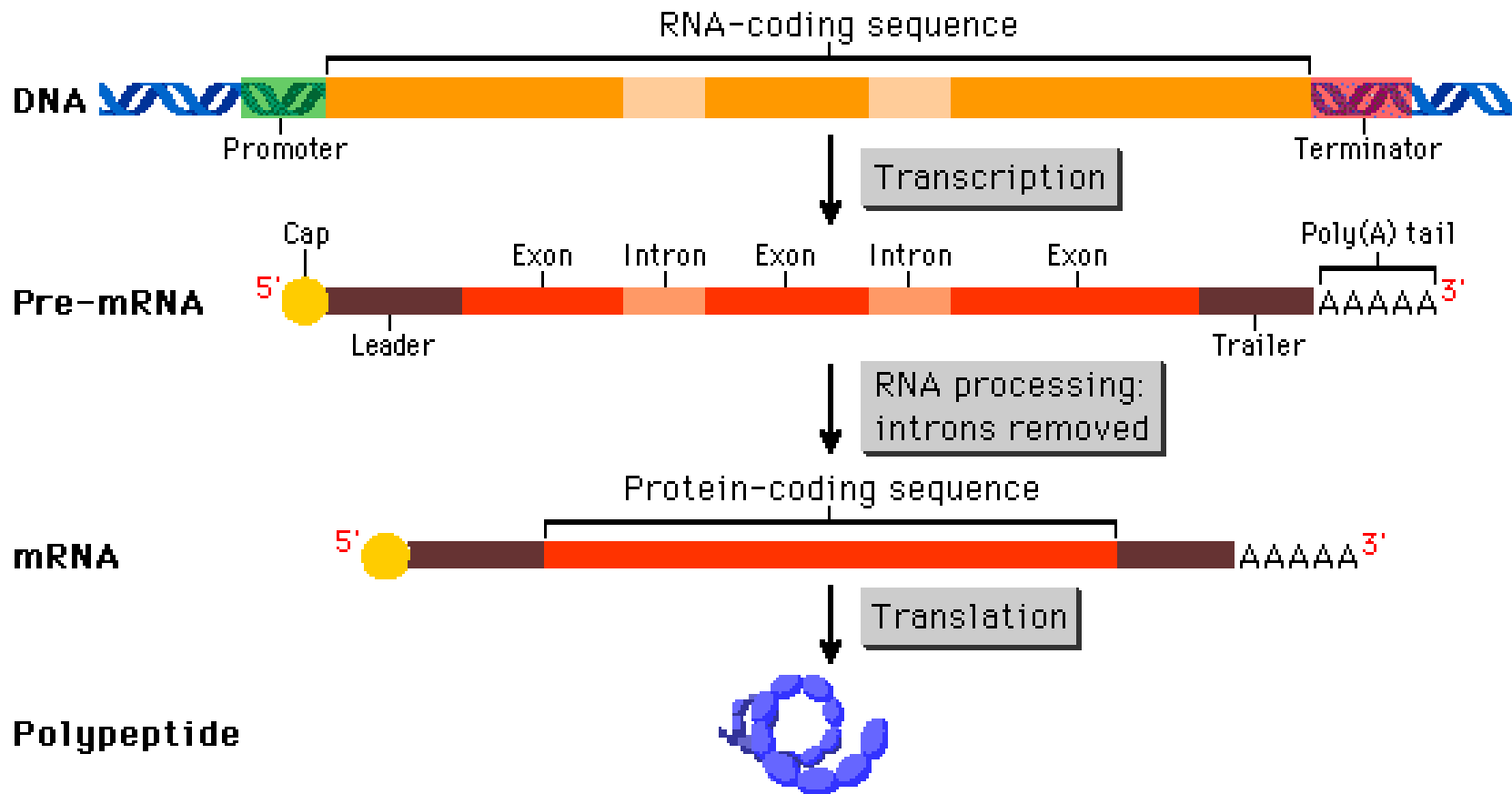


pseudoknot - RNA structure that is minimally composed of two helical segments connected by single-stranded regions or loops

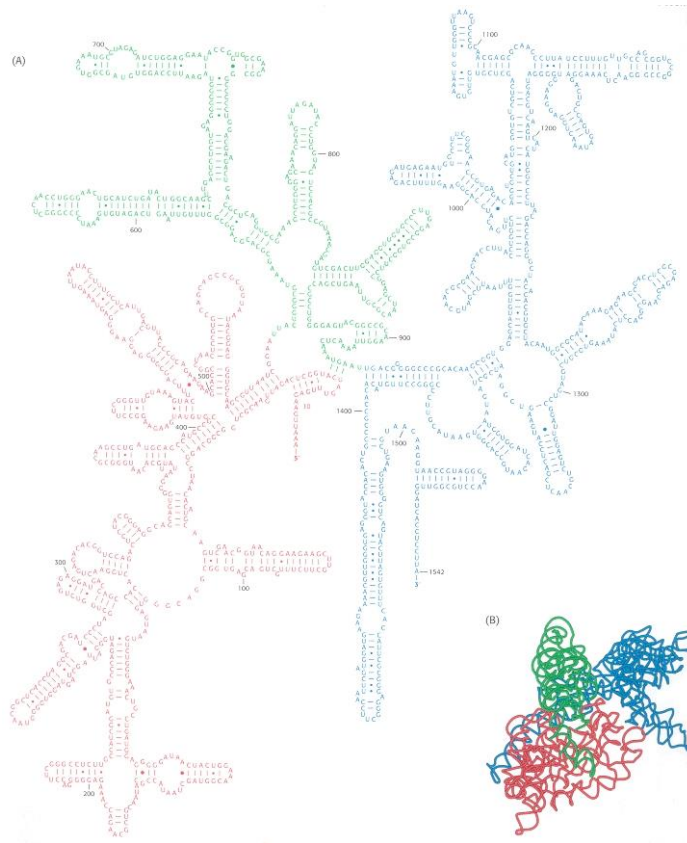


PLoS Biol. 2005 June; 3(6): e213.
 Published online 2005 June 14. doi:
 10.1371/journal.pbio.0030213.

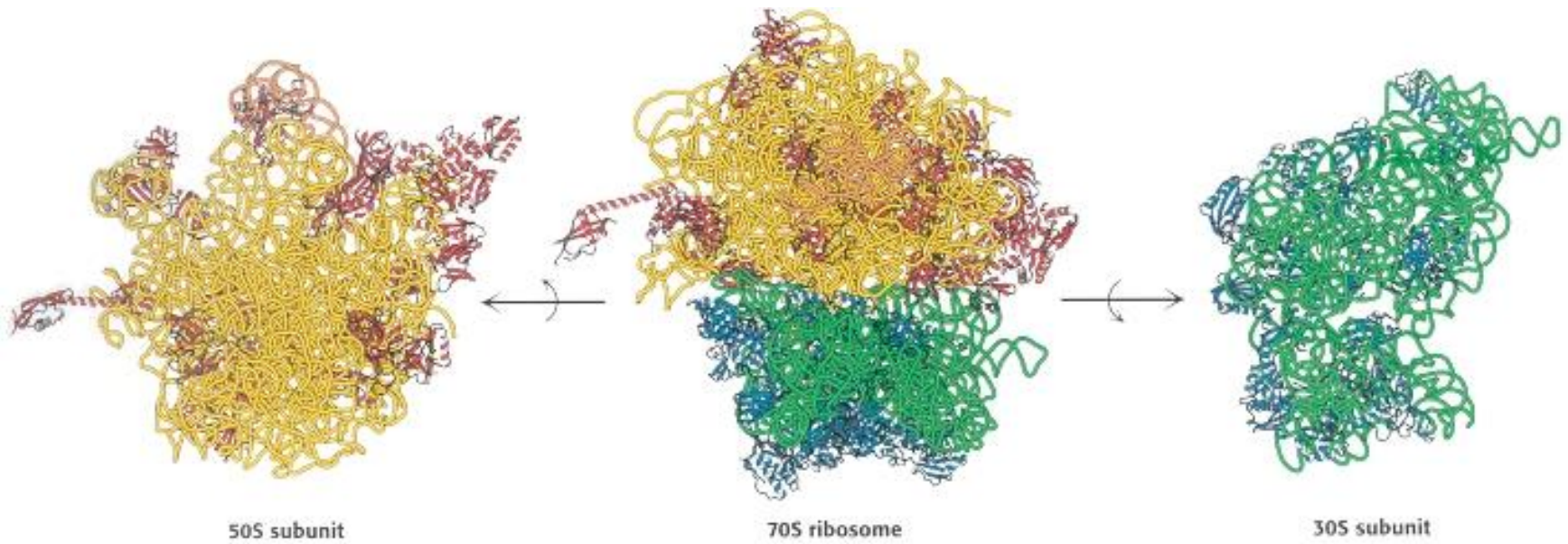
Modifications of mRNA



rRNA folding pattern



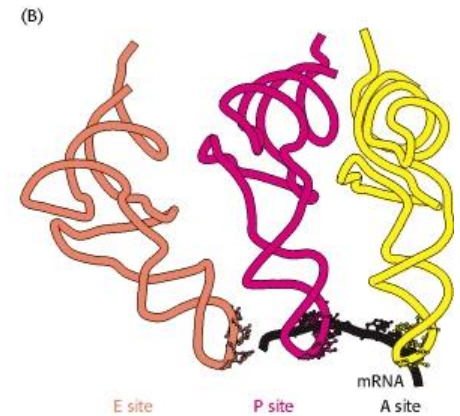
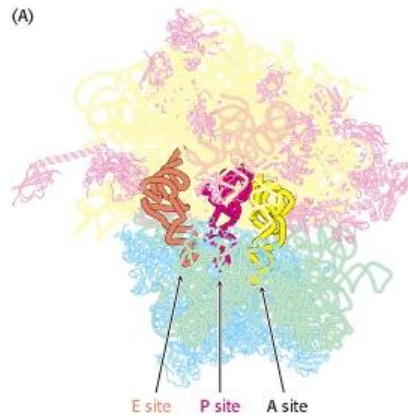
Structure of ribosome

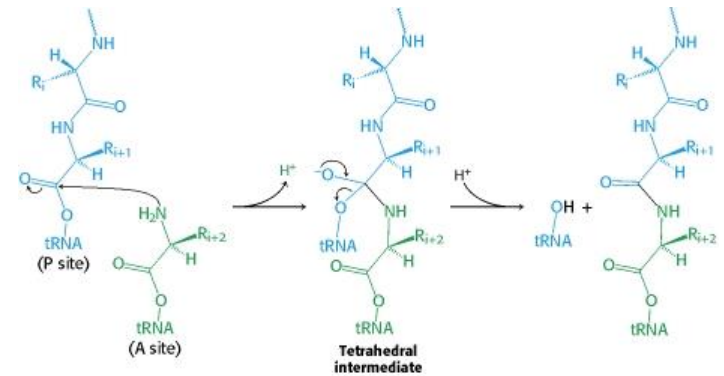
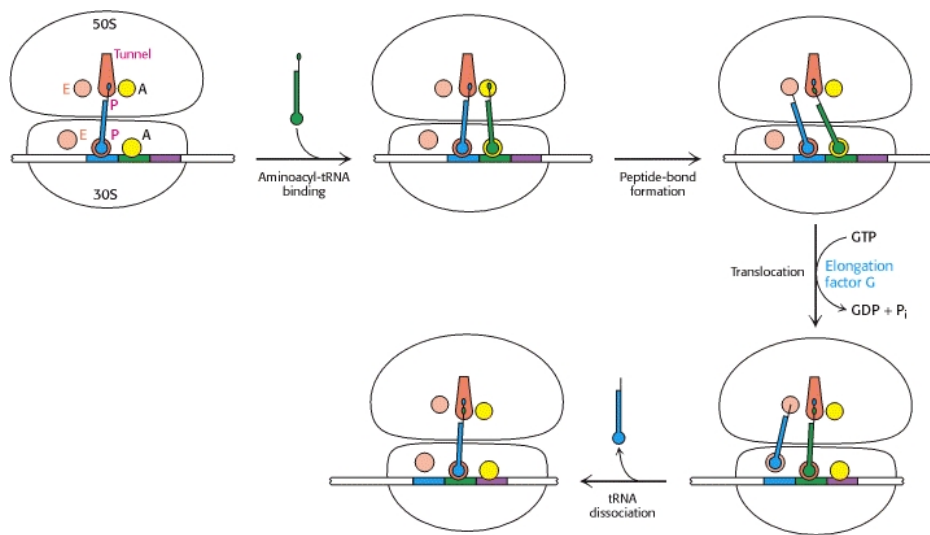


5'	AGCACGAGGGGAAAUCUGAUGGAACGCUAC	3'	<i>E. coli trpA</i>
	UUUGGAU GGAGUGAAA CGAUGGCGAUUGCA		<i>E. coli araB</i>
	GGUAACCAGGUAACAA CCAUGCGAGUGUUG		<i>E. coli thrA</i>
	CAAUUCAGGGUGUGA GAUUGUGAAACCAGUA		<i>E. coli lacI</i>
	AAUCUU GGAGGCUUUUUUUAUGGUUCGUUCU		ϕ X174 phage A protein
	UAACUAAGGAUGAAA AUGCAUGUCUAAGACA		Q β phage replicase
	UCCUAAGGAGGUUUGACCUAUGCGAGCUUUU		R17 phage A protein
	AUGUACUAAGGAGGUUGUAUGGAACAA CGC		λ phage <i>cro</i>

Pairs with 16S rRNA
Pairs with initiator tRNA

Sequences of mRNA initiation sites for protein synthesis in some bacterial and viral mRNA molecules. Comparison of these sequences reveals some recurring features.





Peptide-Bond Formation. The amino group of the aminoacyl-tRNA attacks the carbonyl group of the ester linkage of the peptidyl-tRNA to form a tetrahedral intermediate. This intermediate collapses to form the peptide bond and release the deacylated tRNA

- Genetic code table
- 3 nucleotides encode 1 AA
- Genetic code is nonoverlapping
- Genetic code has no punctuation
- Genetic code is degenerated (64 triplets and 20 AA)
- **Trp** and **Met** - only one codon
- Number of codons correlates with frequency of AA residues in proteins
- Codon is nearly universal (variations in mitochondria and some organisms).
- AUG – start codon (identical for Met), in prokaryotes additional information about the start codon is provided by Shine Dalgarno sequence, in eukaryotes AUG close to 5' end of mRNA is the start codon
- UAA, UAG, UGA –stop codons recognized by release factors

		Second position					
		U	C	A	G		
First position	U	phenyl-alanine	serine	tyrosine	cysteine	U	
				leucine	stop	stop	C
		leucine		stop	stop	A	
				stop	tryptophan	G	
	C	leucine	proline	histidine	arginine	U	
				glutamine		C	
		leucine		glutamine		A	
				glutamine		G	
	A	isoleucine	threonine	asparagine	serine	U	
				lysine	arginine	C	
		methionine		lysine	arginine	A	
				lysine	arginine	G	
G	valine	alanine	aspartic acid	glycine	U		
			glutamic acid		C		
	valine		glutamic acid		A		
			glutamic acid		G		

Fungi

Candida cylindrica
CTG = Ser

Algae

TAA = Gln
TAG = Gln

Tetrahymena, Paramecium

TAA = Gln
TAG = Gln

Third position