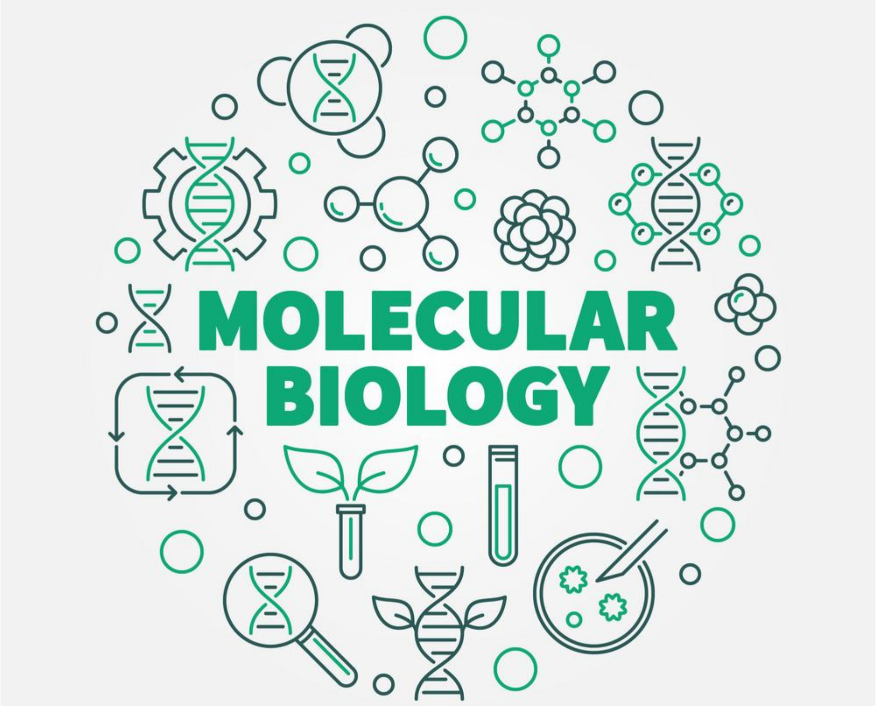
Molecular Biology

- **College of Education for Pure Science**
- **Dep. Of Biology**
- 4th Stage (First semester)



MOLECULAR BIOLOGY

is the branch of biology that studies the molecular basis of biological activity.

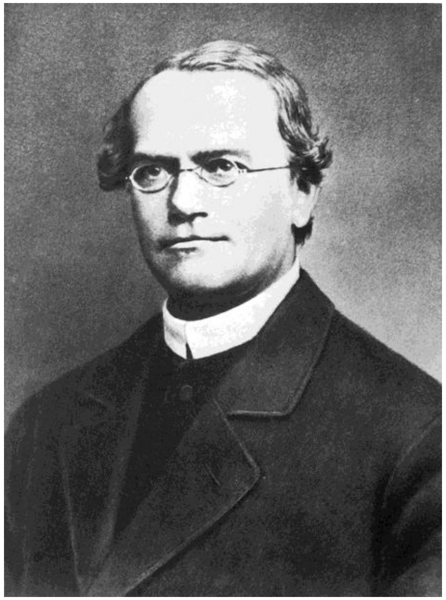
Living things are made of chemicals just as non-living things are, so a molecular biologist studies how molecules interact with one another in living organisms to perform the functions of life.

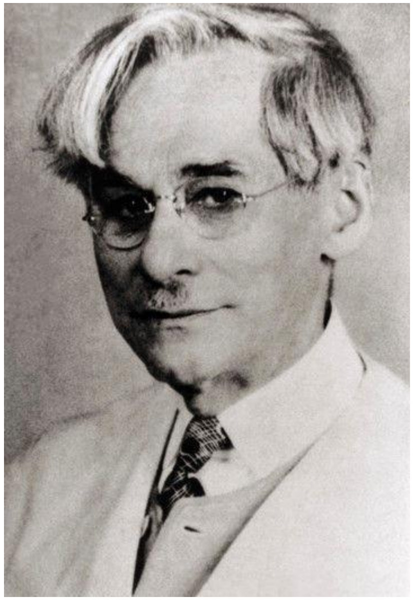
Molecular biologists conduct experiments to investigate the structure, function, processing, regulation and evolution of biological molecules and their interactions with one another — providing micro-level insights into how life works.

The field of molecular biology studies macromolecules and the macromolecular mechanisms found in living things, such as the molecular nature of the gene and its mechanisms of gene replication, mutation, and expression

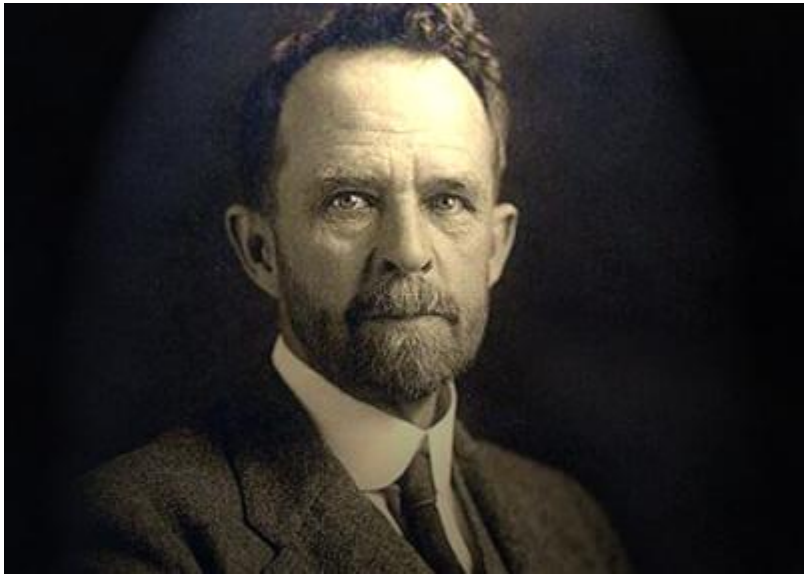
History of Molecular Biology

- Despite its prominence in the contemporary life sciences, molecular biology is a relatively young discipline, originating in the 1930s and 1940s, and becoming institutionalized in the 1950s and 1960s.

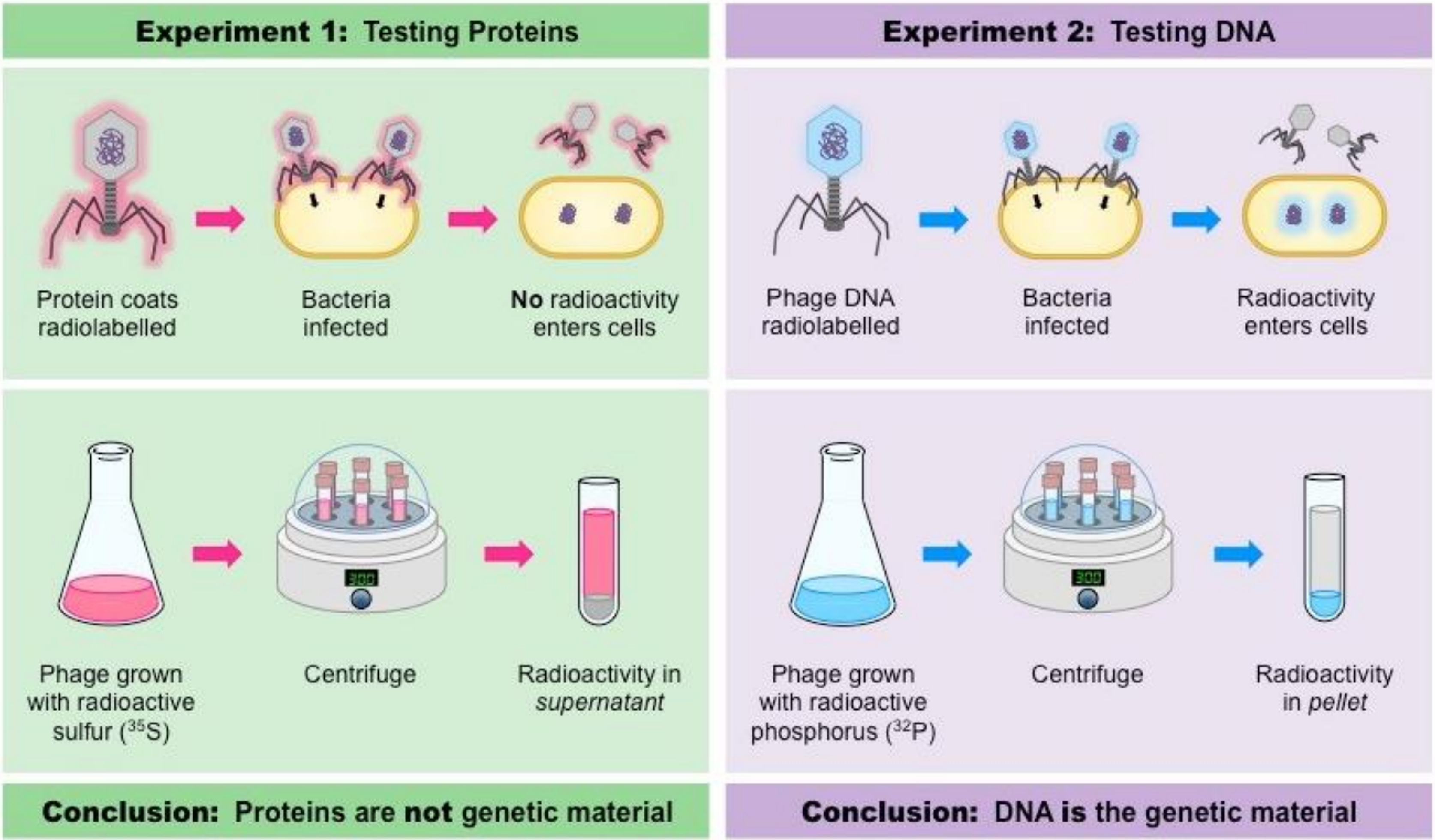




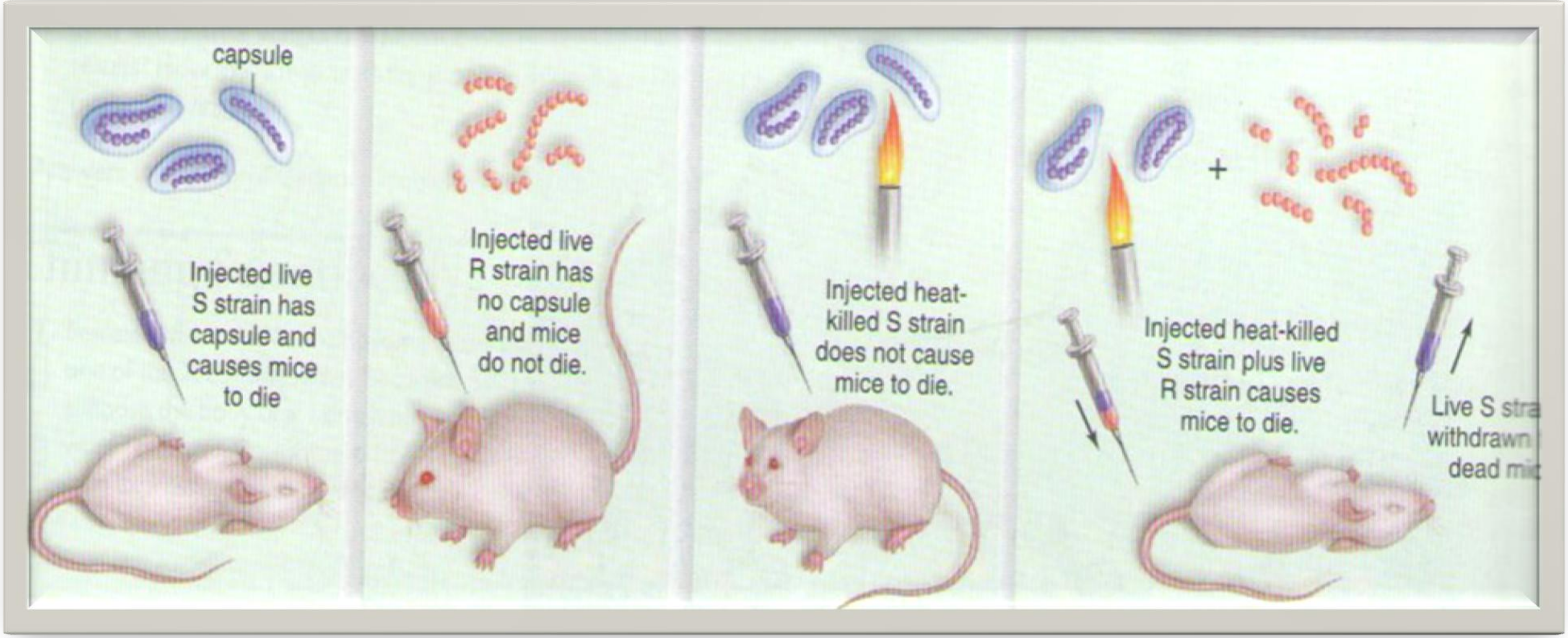
- It should not be surprising, then, that many of the philosophical issues in molecular biology are closely intertwined with this recent history. This section sketches four facets of molecular biology's development: its origins, its classical period, its subsequent migration into other biological domains, and its more recent turn to genomics and post-genomics. The rich historiography of molecular biology can only be briefly utilized in this shortened history



Summary of the Hershey-Chase Experiment



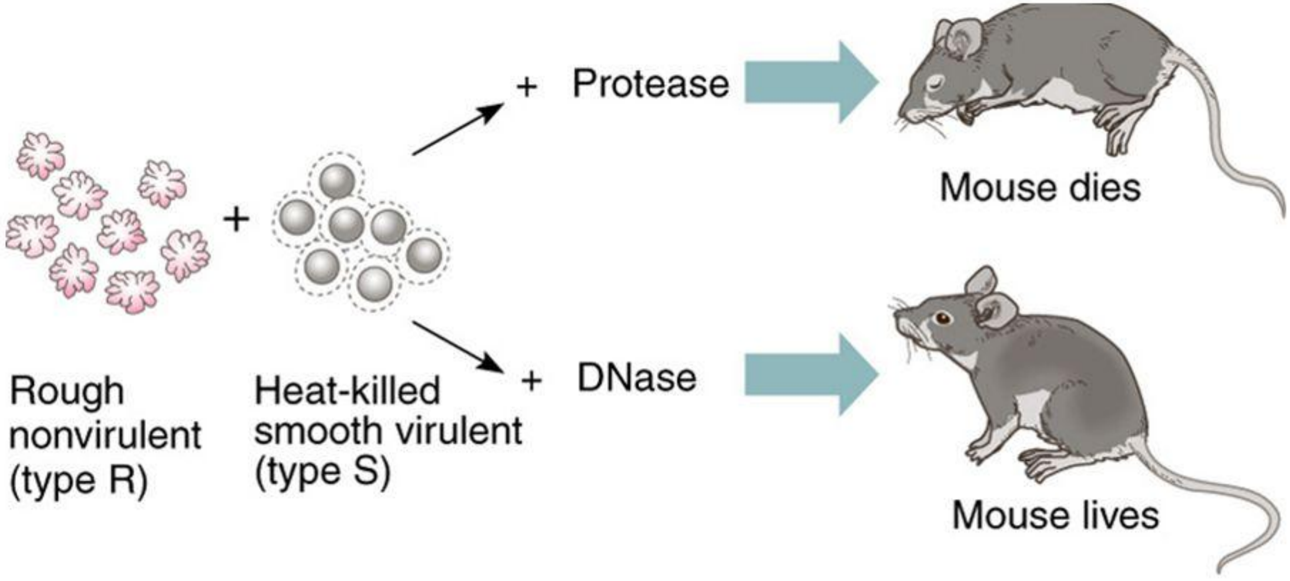
Transformation of Bacteria



Avery, MacLeod, McCarty

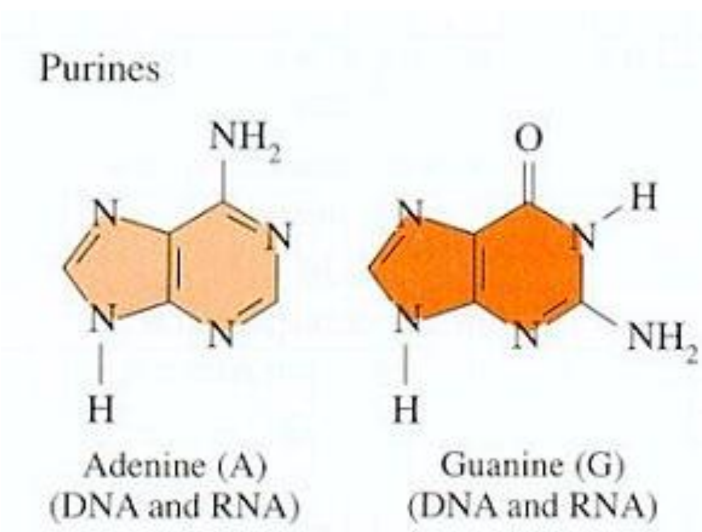
1944

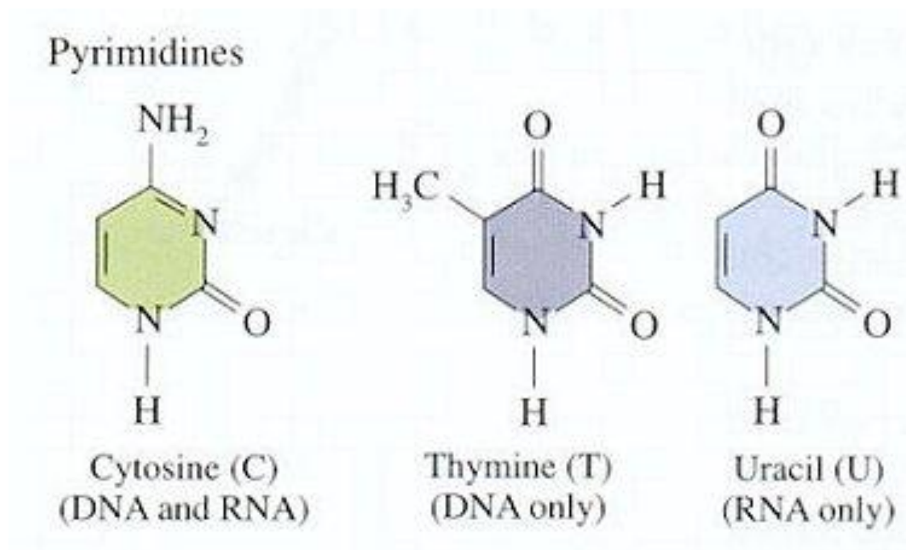
- DNase vs. Protease



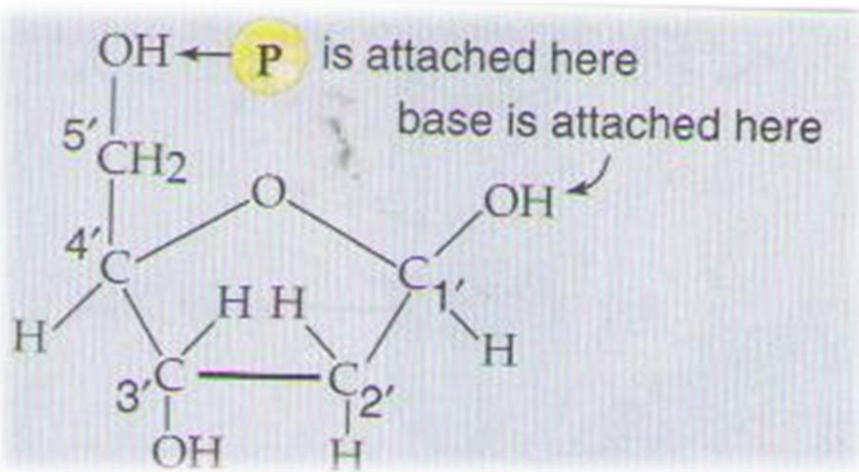
DNA Structure

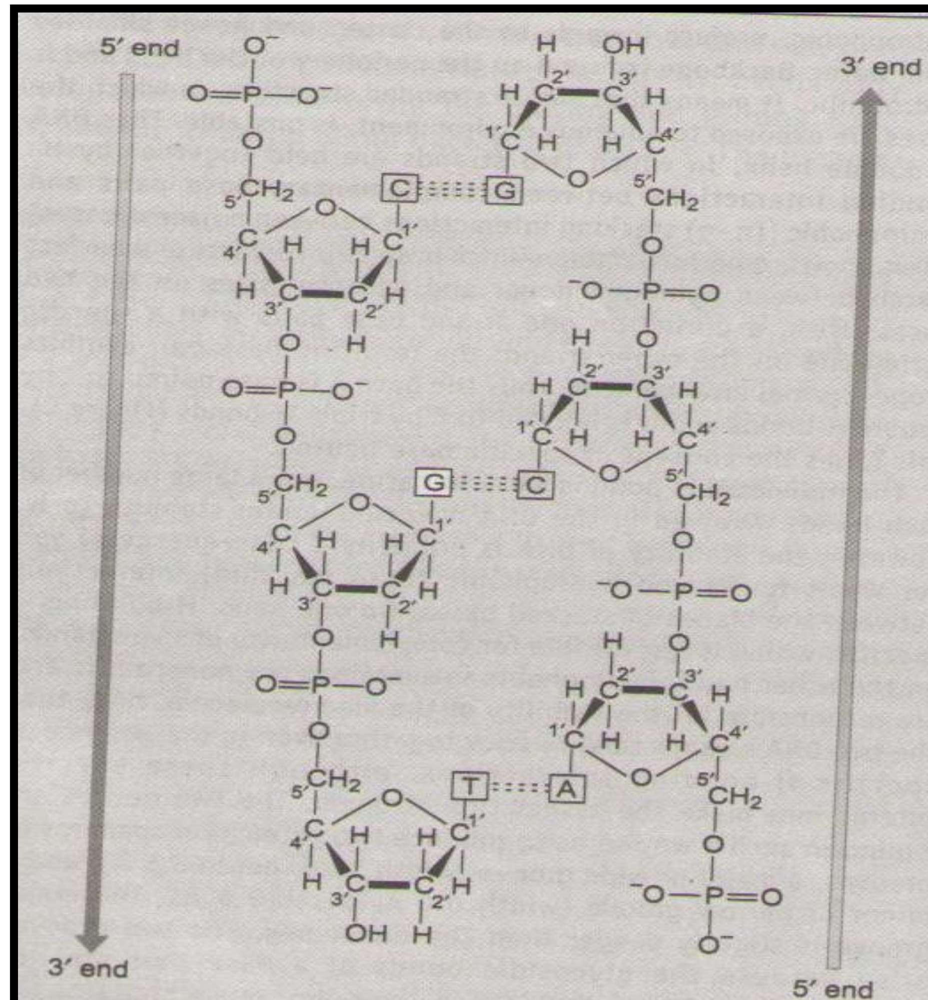
- DNA is the chemical name for the molecule that carries genetic instructions in all living things. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands are held together by bonds between the bases; adenine bonds with thymine, and cytosine bonds with guanine.



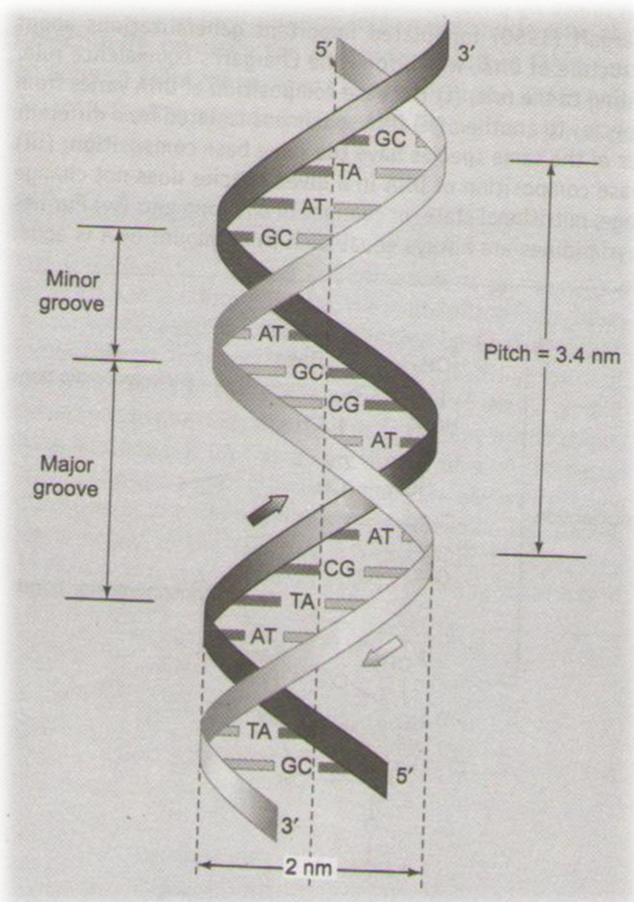


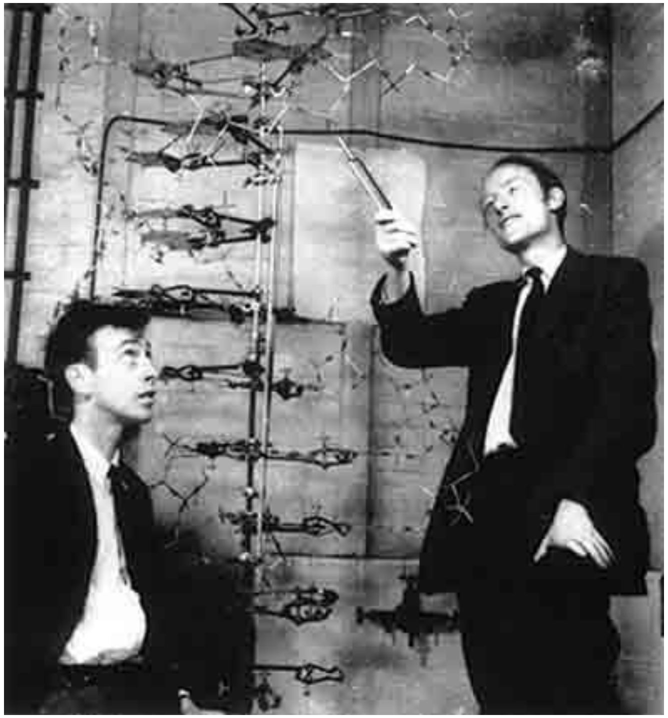
DNA Structure



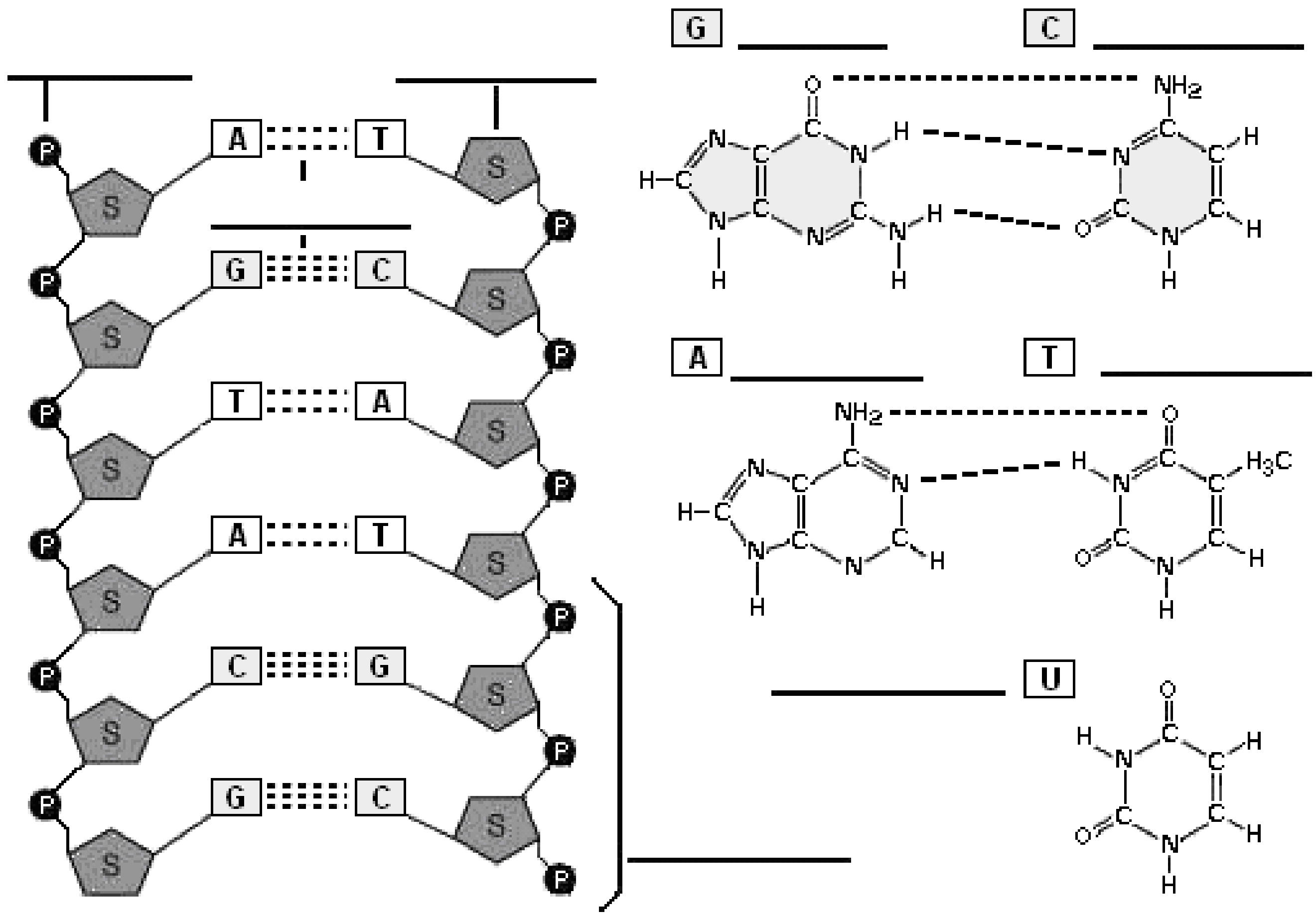


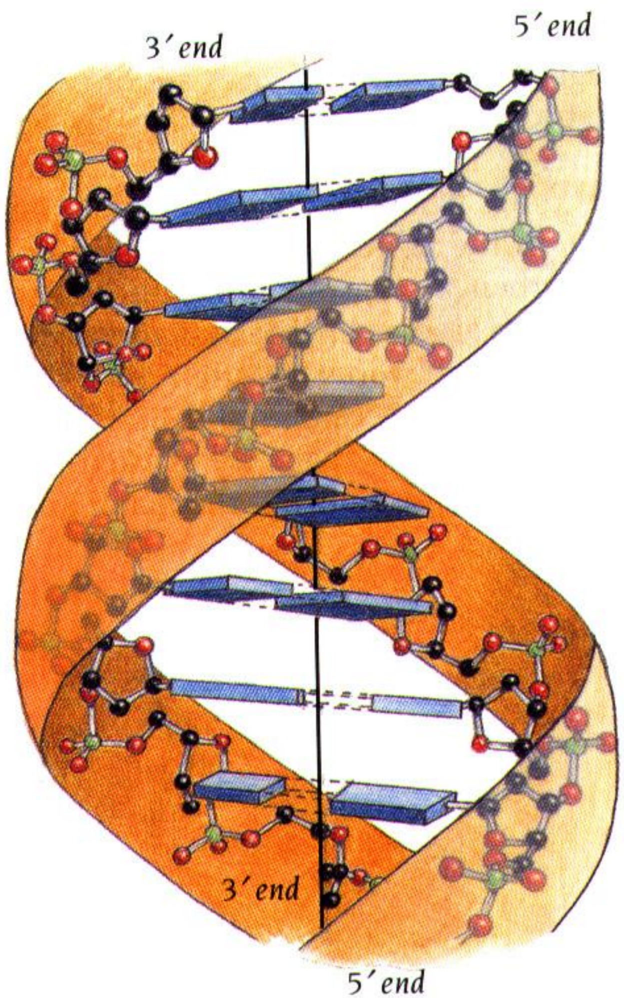
Double helix for DNA

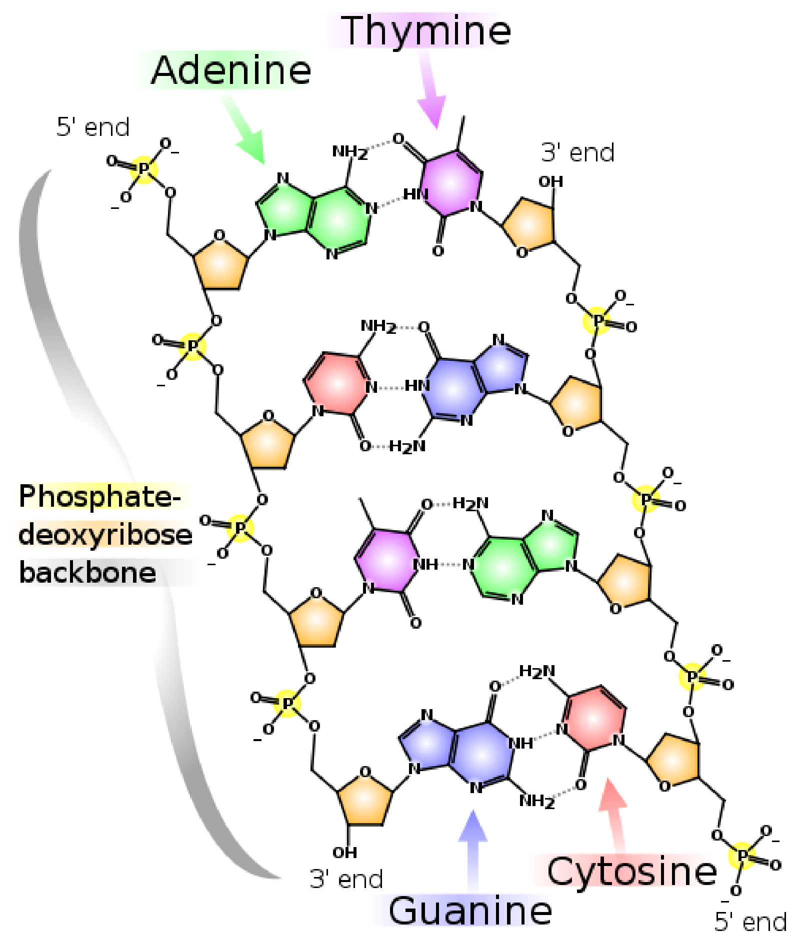




Deoxyribonucleic Acid (DNA)



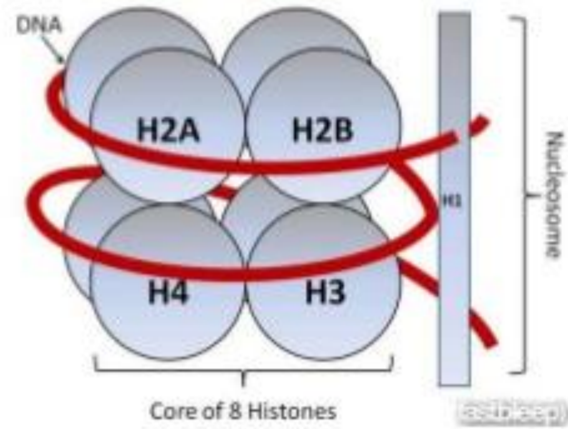

3′ end
5′ end
3′ end
5′ end


Thymine
Adenine
5' end
3' end
Phosphate-
deoxyribose
backbone
3' end
Cytosine
Guanine
5' end

2.6.4 Crick and Watson's elucidation of the structure of DNA using model making.

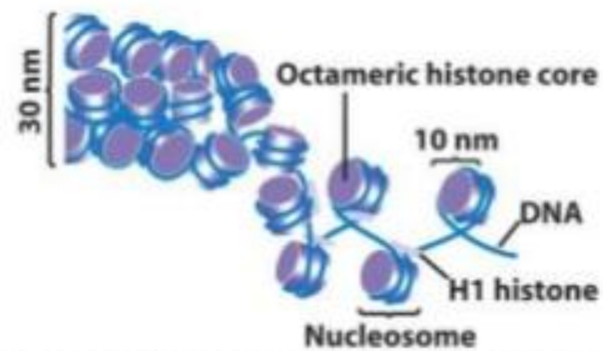
Nucleosomes

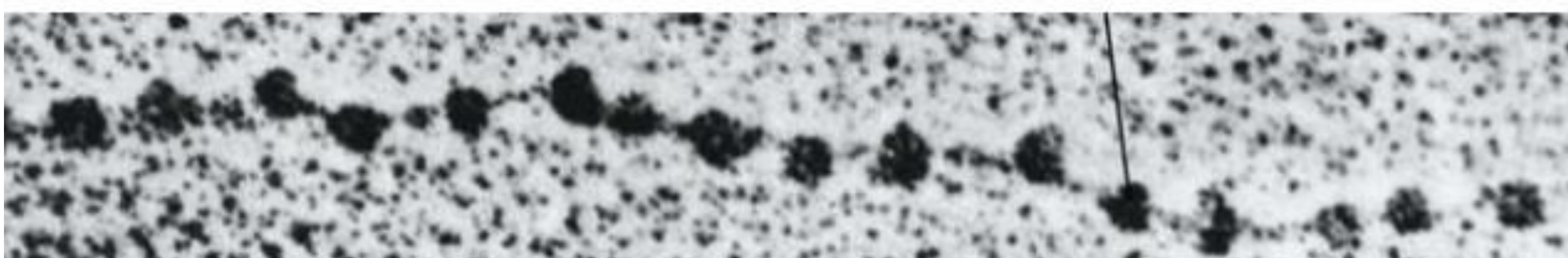
- DNA in eukaryotes is associated with proteins called histones.
- The octamer & DNA combination is attached to an H1 histone, forming a nucleosome.
- The nucleosome serves to protect the DNA from damage and to allow long lengths of DNA to be supercoiled



Supercoiling

- Supercoiling allows the chromosomes to be mobile in mitosis & meiosis.
- Supercoiled DNA ***cannot*** be transcribed for protein synthesis. Allows genes to be switched ON and OFF.

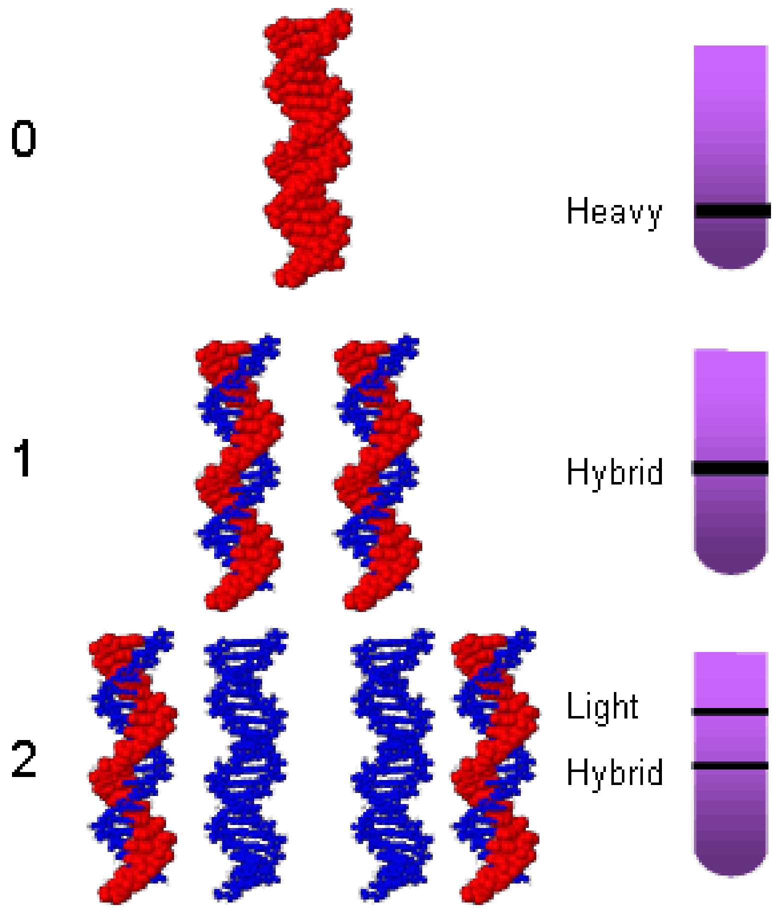


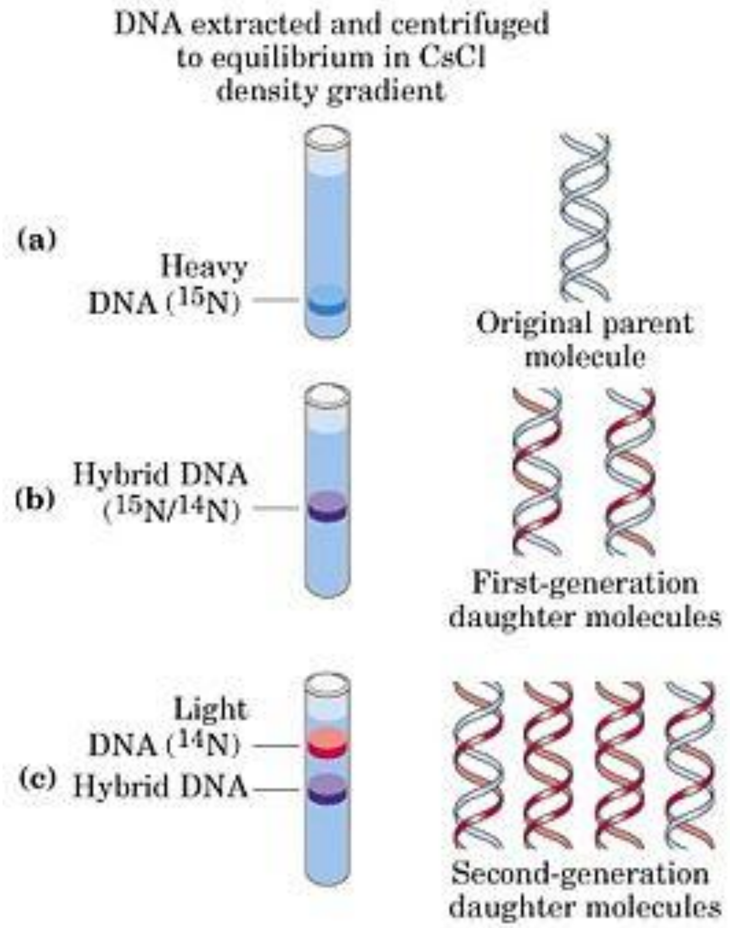


DNA Replication

Meselson and Stahl experiment

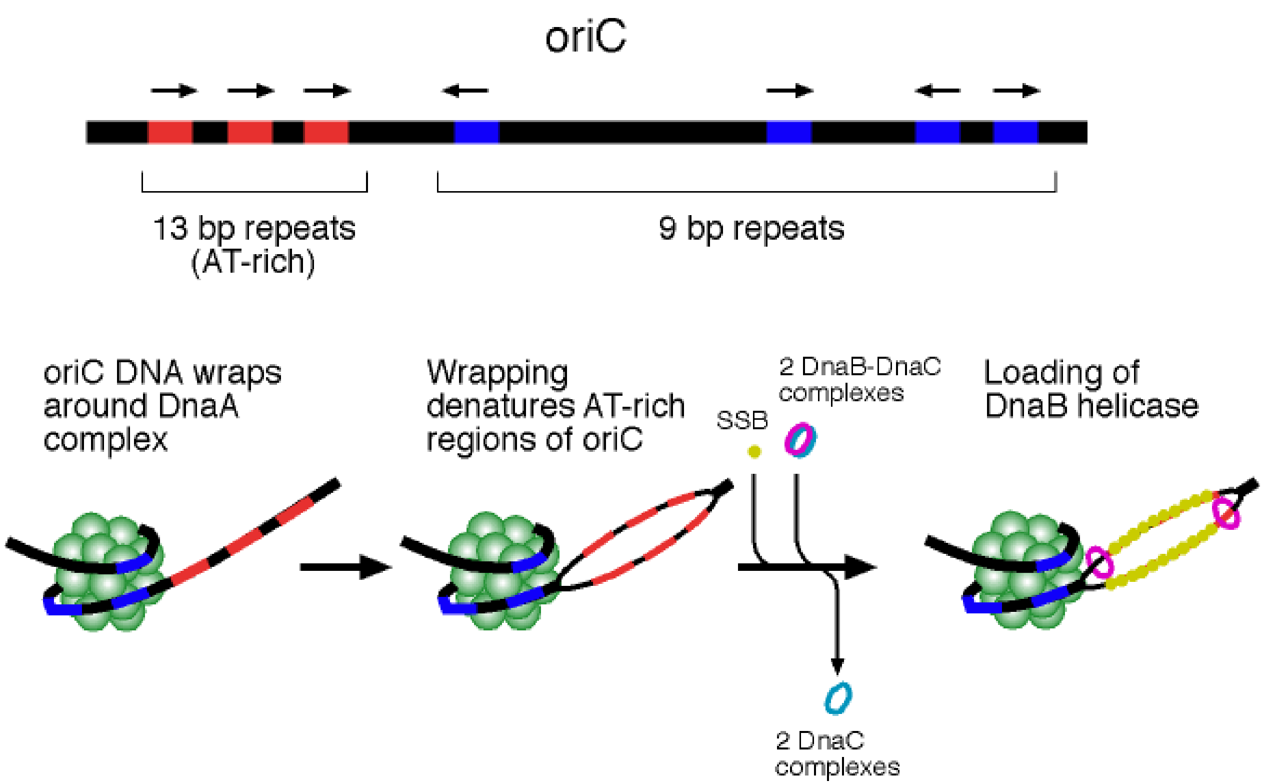
- Matt Meselson and Frank Stahl designed **the most beautiful experiment in biology.**
- The experiment tests the DNA replication models.
- They used bacteria grown in a media of a heavy isotope of nitrogen .


0
Heavy
1
Hybrid
2
Light
Hybrid


DNA extracted and centrifuged to equilibrium in CsCl density gradient
(a)
Heavy DNA (^{15}N)
Original parent molecule
(b)
Hybrid DNA ($^{15}N/^{14}N$)
First-generation daughter molecules
(c)
Light DNA (^{14}N)
Hybrid DNA
Second-generation daughter molecules

- Replication of DNA

Initiation of replication
Elongation of Replication
Termination of Replication



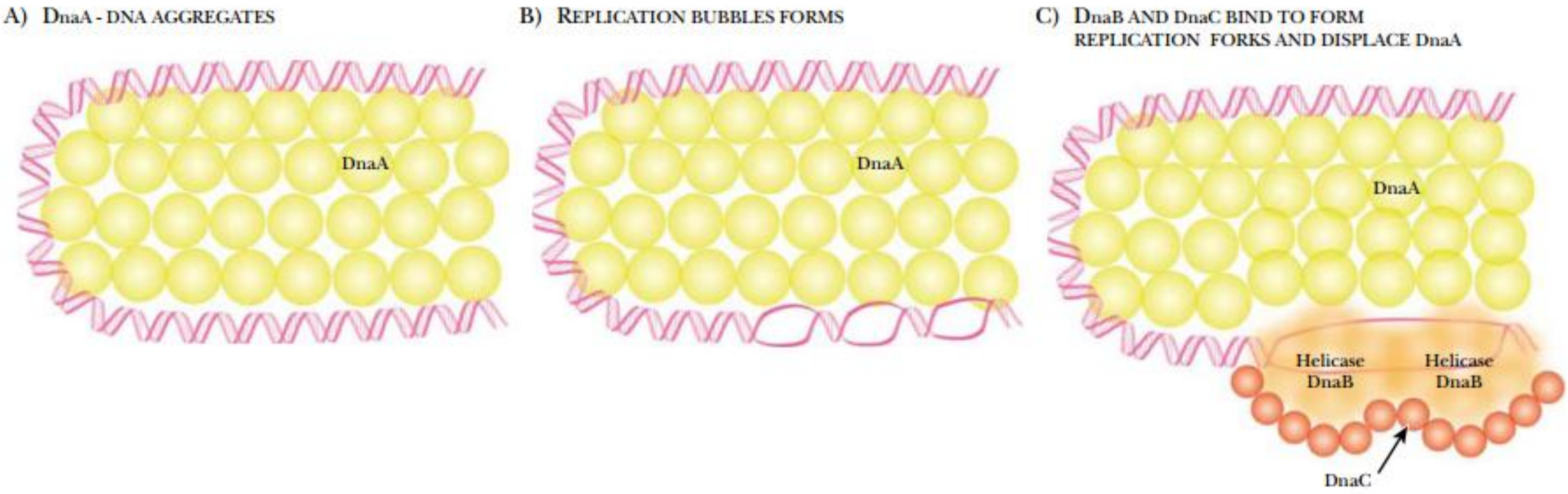
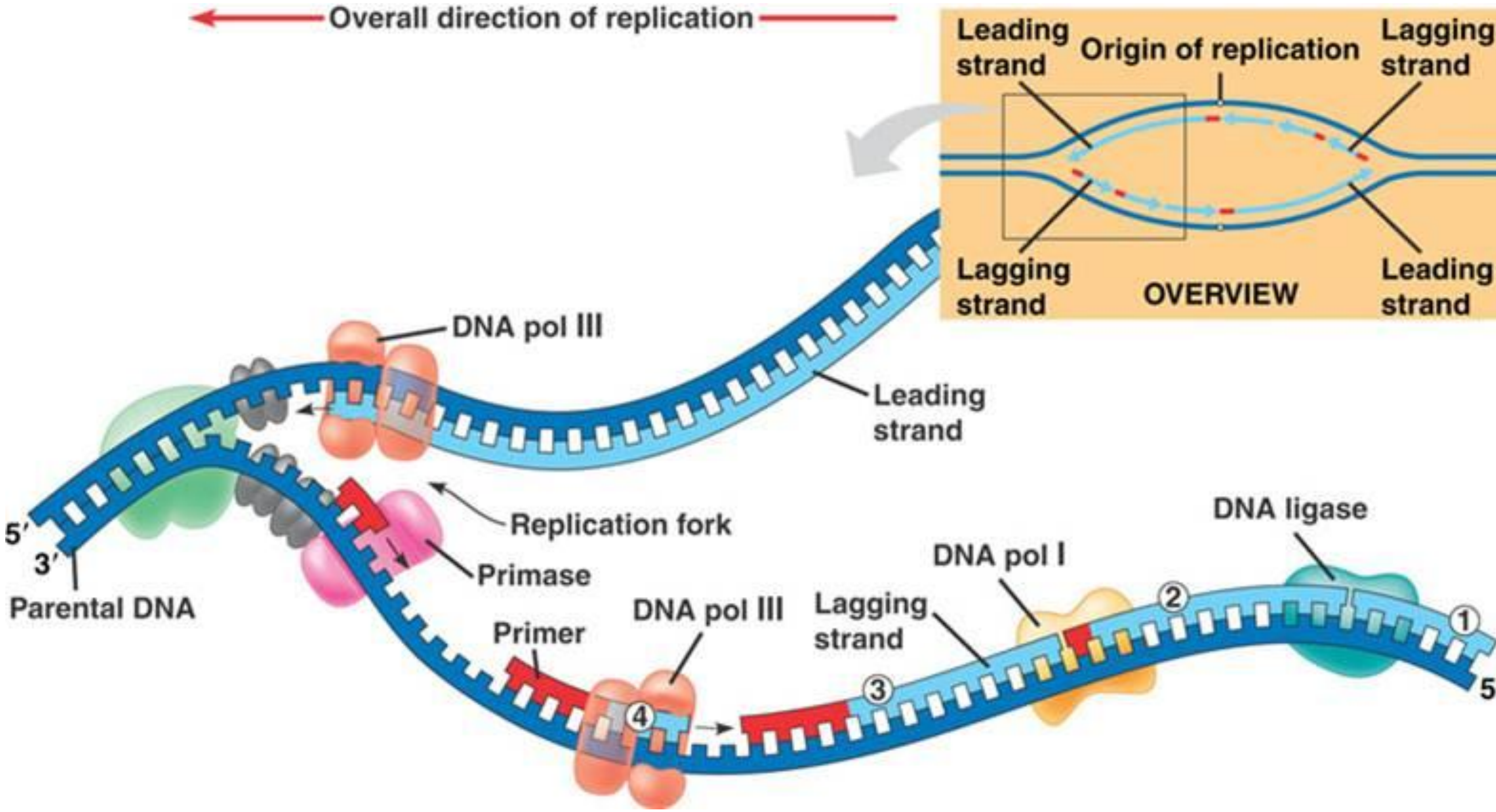


FIGURE 5.19 ***Three Steps in the Initiation of Replication by DnaA***

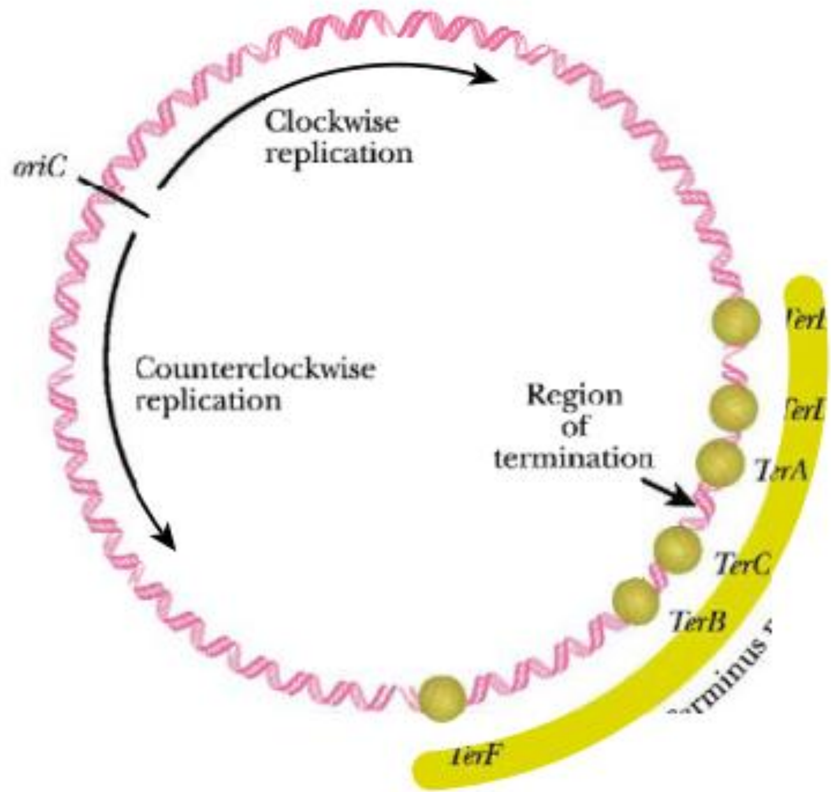
A) DnaA protein binds first to the four nine-base repeats, and then to the three 13-base repeats. B) As more DnaA binds, the DNA folds and the three 13-base repeats are unwound. C) Two complexes of DnaB and DnaC bind to the three 13-base repeats. This pushes DnaA away and causes the DNA strand to open all along the AT-rich region. The two DnaB complexes now start two replication forks, each headed in opposite directions around the circular DNA.



Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
DNA pol III
Leading strand
Replication fork
Primase
5′
3′
Parental DNA
Primer
DNA pol III
Lagging strand
DNA pol I
DNA ligase
4
3
2
1
3′
5′

FIGURE 5.22 ***Termination of replication by Tus and Ter Sites***

The circular bacterial chromosome has a termination region, or terminus, with several sites that stop replication forks moving clockwise (*TerF, TerB* and *TerC*) and counterclockwise (*TerE, TerD* and *TerA*).



DNA-pol of eukaryotes

DNA-pol α: initiate replication and synthesize primers → DnaG, primase

DNA-pol β: replication with low fidelity → repairing

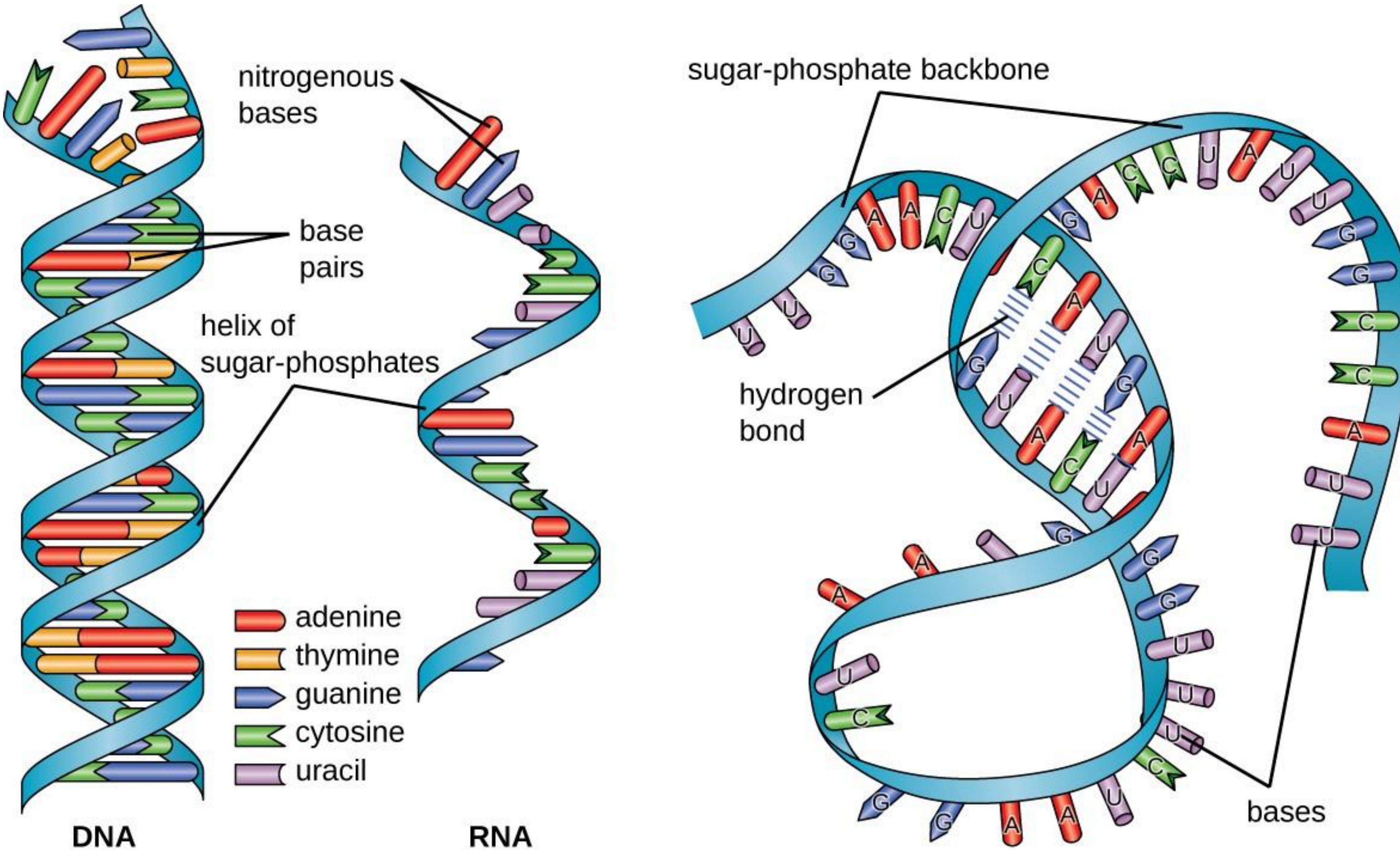
DNA-pol γ: polymerization in mitochondria

DNA-pol δ: elongation → DNA-pol III

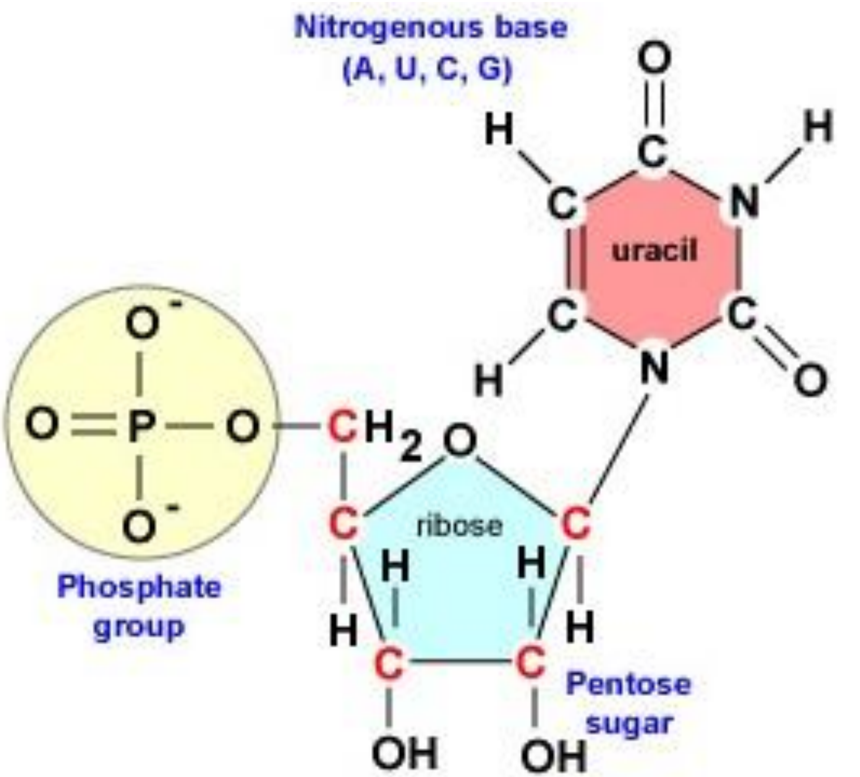
DNA-pol ε: proofreading and filling gap → DNA-pol I

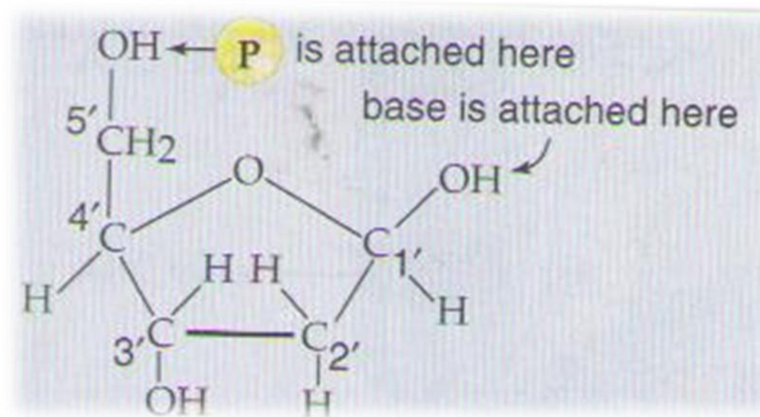
Difference between Prokaryotic and Eukaryotic Replication

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
DNA polymerase types	5	14
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	Pol δ, pol ε

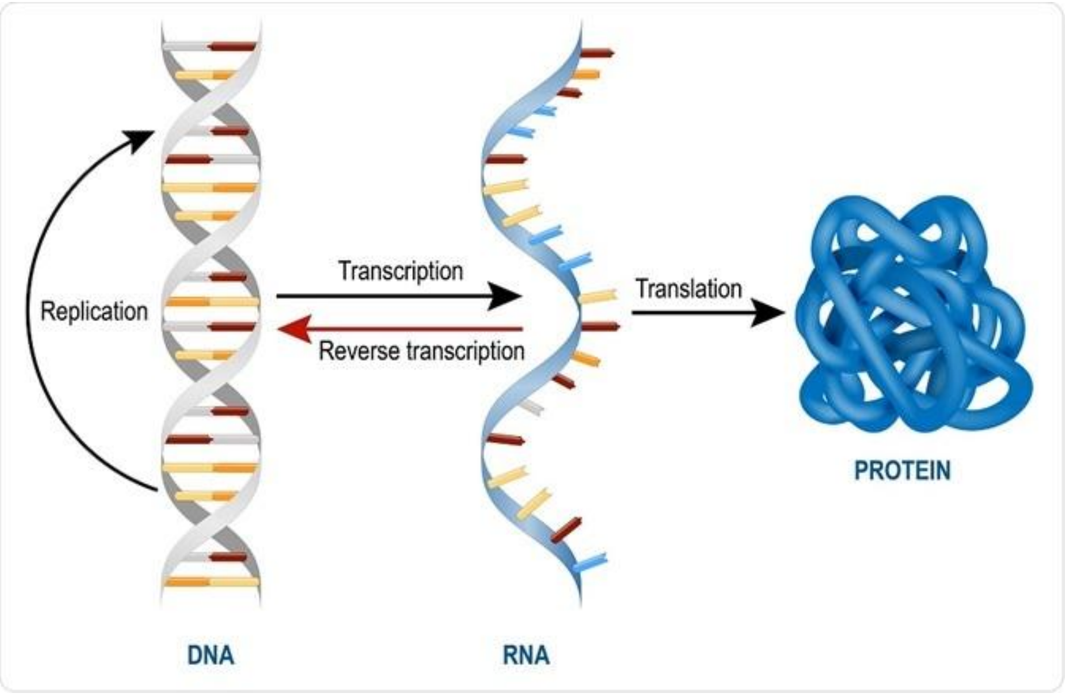

nitrogenous bases
base pairs
helix of sugar-phosphates
adenine
thymine
guanine
cytosine
uracil
DNA
RNA
sugar-phosphate backbone
hydrogen bond
bases

(a) (b)

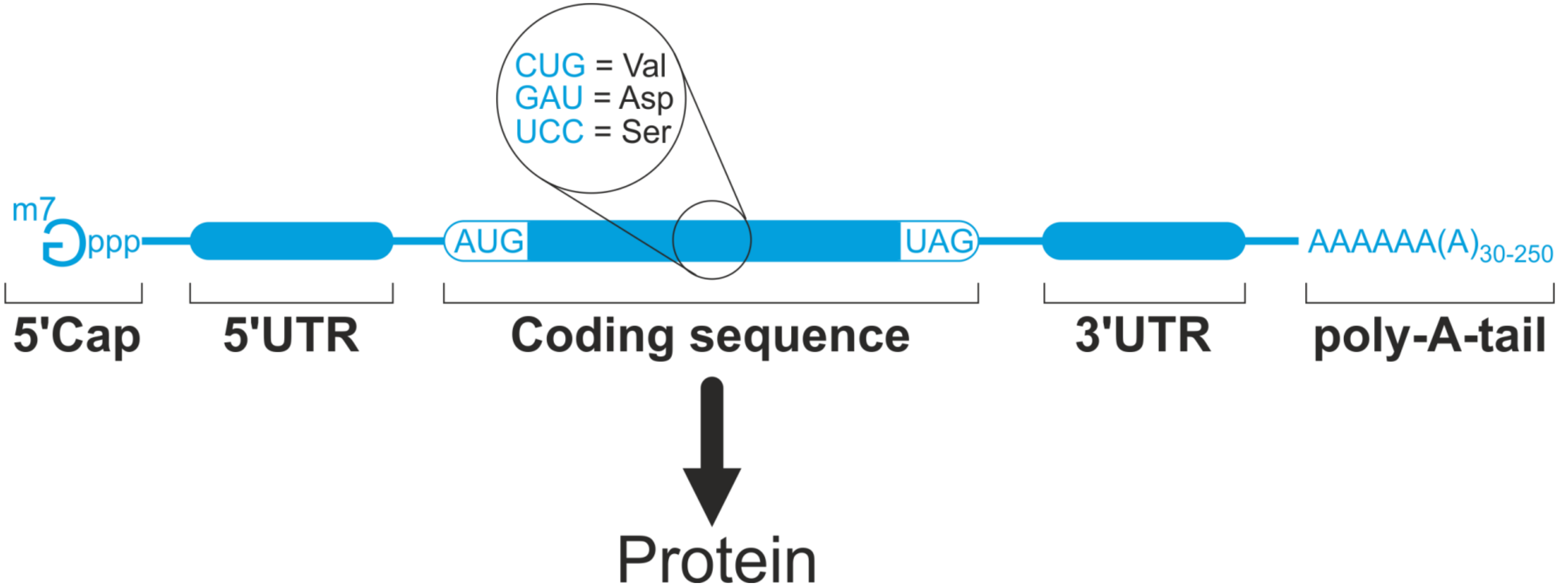

Nitrogenous base
(A, U, C, G)
uracil
ribose
Phosphate
group
Pentose
sugar


P is attached here
base is attached here
5′
4′
3′
2′
1′

mRNA



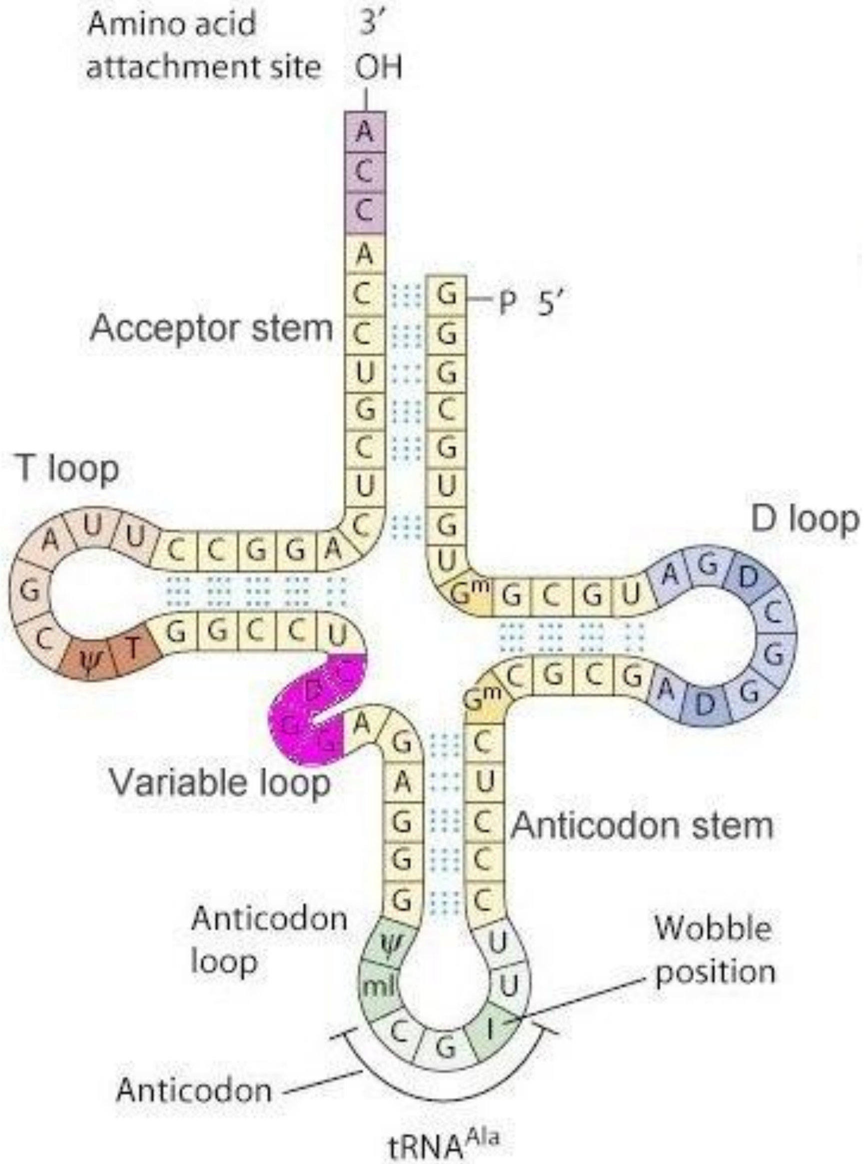
Replication
Transcription
Reverse transcription
Translation
DNA
RNA
PROTEIN



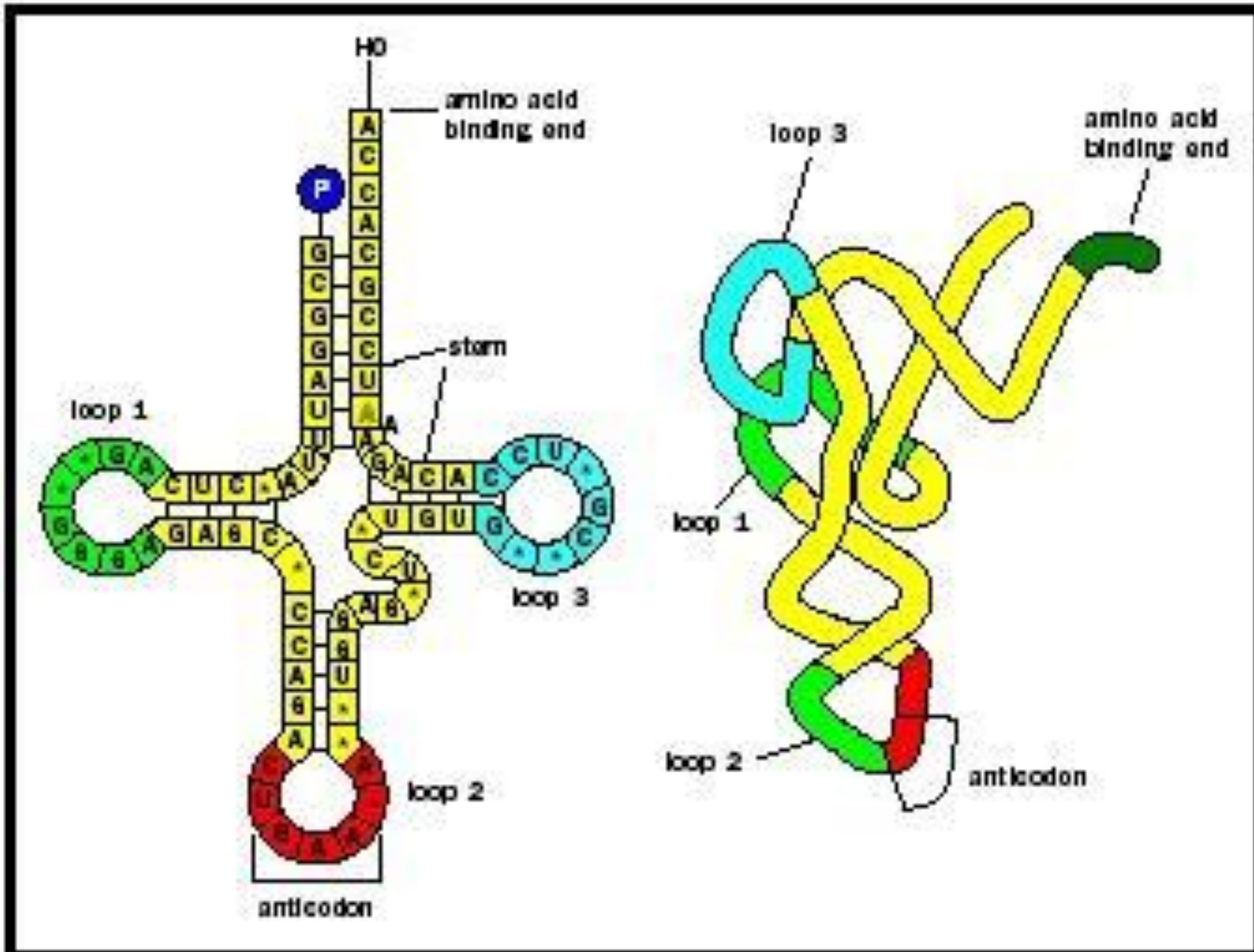
CUG = Val
GAU = Asp
UCC = Ser
m7
Gppp
AUG
UAG
AAAAAA(A)30-250
5'Cap
5'UTR
Coding sequence
3'UTR
poly-A-tail
Protein

tRNA

- t-RNA (transfer RNA) is also named as S-RNA (soluble or supernatant RNA) and adaptor RNA.
- t-RNA is a family of nearly 60 small sized ribonucleic acids.
- 10 – 15% of total cellular RNA is t-RNA.
- t-RNAs are small molecules with about 74 – 95 ribonucleotides.
- Sedimentation constant – 3.8S
- Molecular weight – nearly 25,000 – 30,000 Dalton
- t-RNAs are made up of a single stranded polynucleotide chain

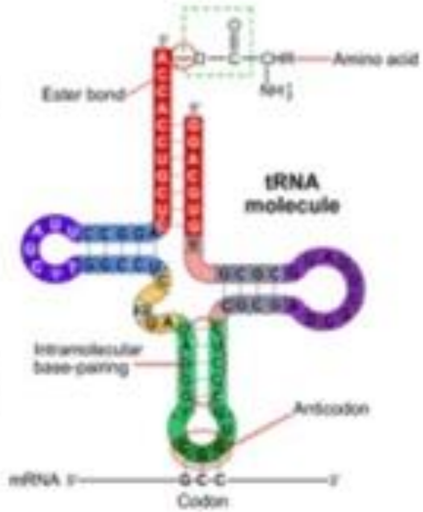


Amino acid attachment site
3′ OH
Acceptor stem
P 5′
T loop
D loop
Variable loop
Anticodon stem
Anticodon loop
Wobble position
Anticodon
tRNA^Ala


HO
amino acid binding end
P
stem
loop 1
loop 3
loop 2
anticodon
loop 3
amino acid binding end
loop 1
loop 2
anticodon

mRNA vs tRNA

http://tinyurl.com/32zyfj5

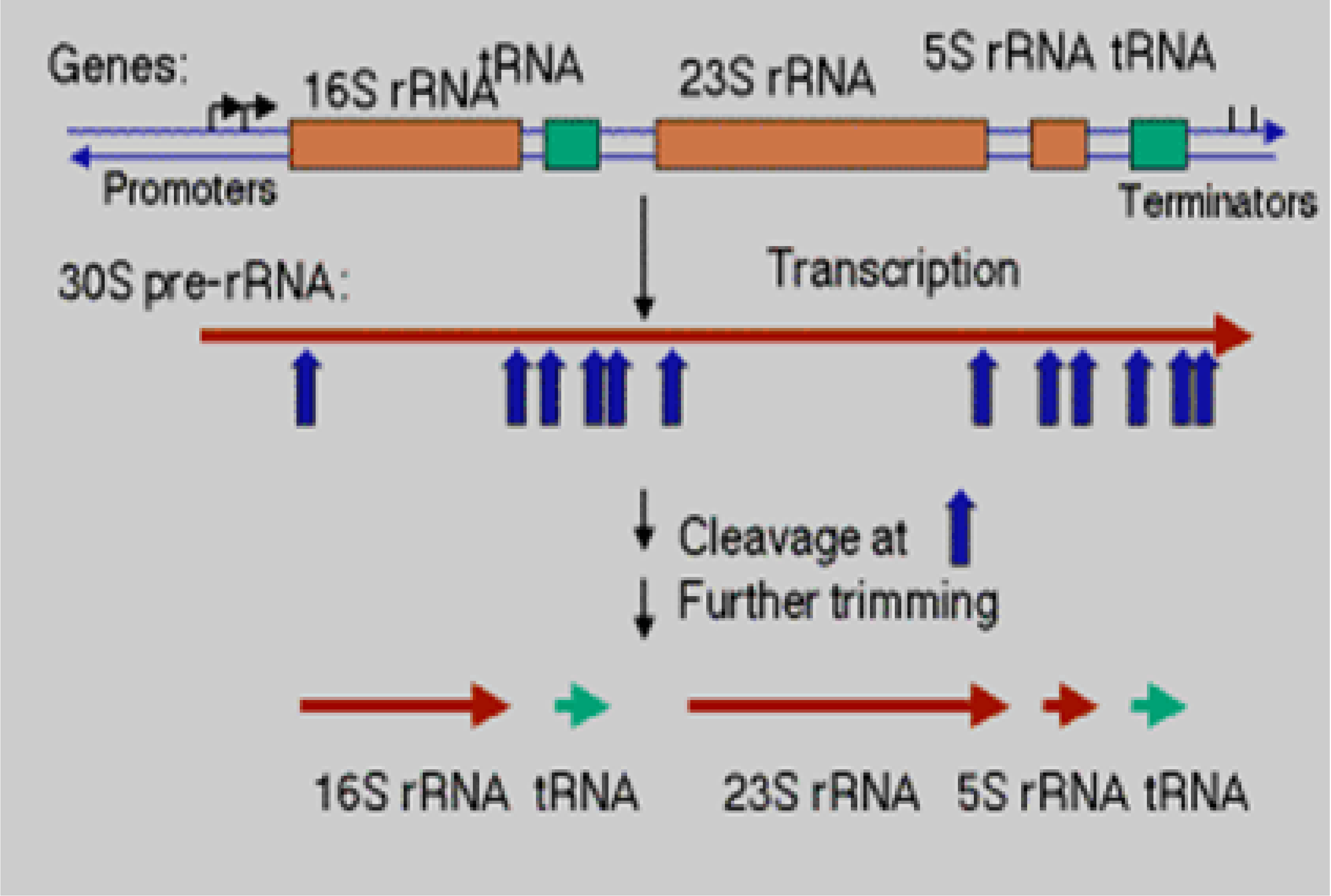


http://tinyurl.com/25oj4ho

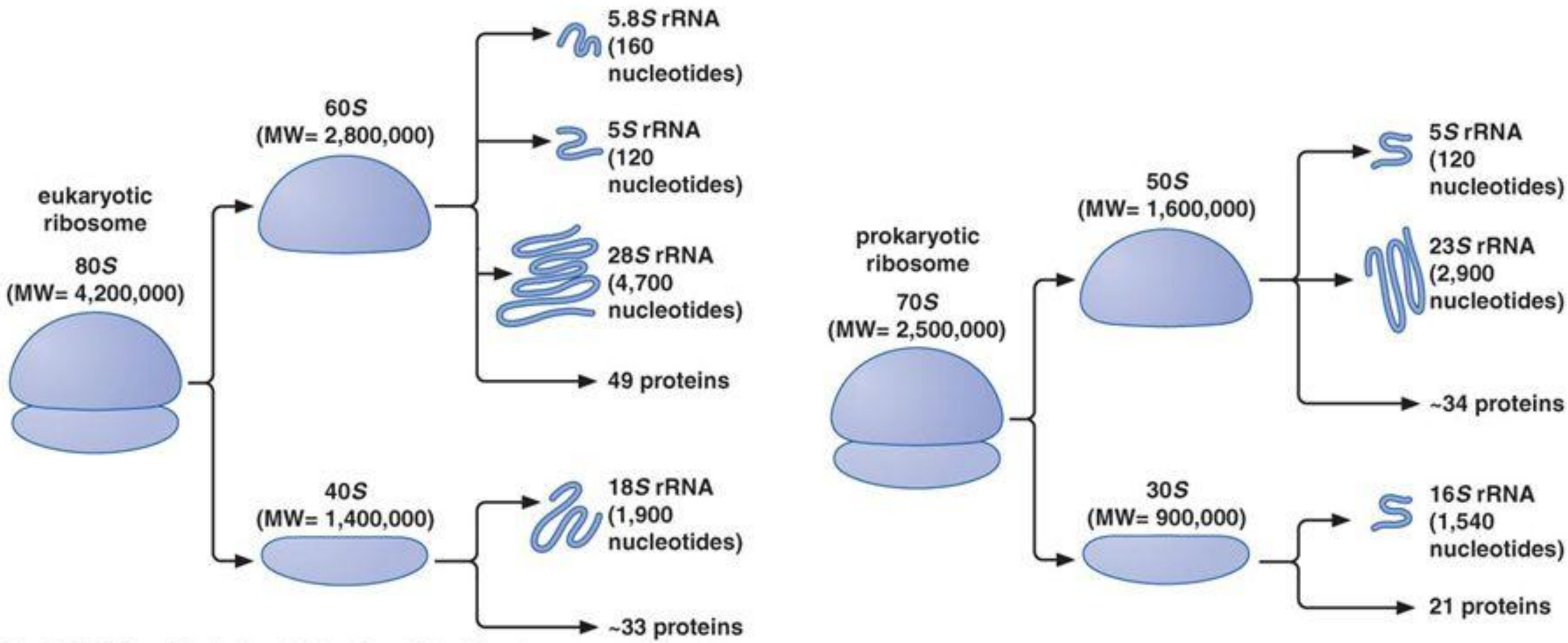
Both use A, U, G and C bases

m = messenger	t = transfer
transcribed from DNA in the nucleus and posted out to the ribosomes for translation	used to translate mRNA by the ribosomes, making a new polypeptide
codons are complementary to DNA triplets	anticodons are complementary to mRNA codons and correspond to specific amino acids
mRNA is a simple strand	tRNA has a clover-shaped loop structure
broken down after translation	reactivated with a new amino acid after translation

A gene is made up of triplets of DNA bases, coding for specific amino acids. DNA in the nucleus is transcribed to an mRNA strand, using complementary base pairing to convert the triplets of DNA to the codons of mRNA - and the base U is used instead of T. This is posted out of the nucleus to the ribosomes. Ribosomes move along the mRNA translating it to a polypeptide. To do this, the anticodons of tRNA bind to the codons on mRNA (again by complementary base pairing), carrying their specific amino acid. A condensation reaction joins neighbouring amino acids, making a polypeptide chain.


Genes:
16S rRNA
tRNA
23S rRNA
5S rRNA
tRNA
Promoters
Terminators
30S pre-rRNA:
Transcription
Cleavage at
Further trimming
16S rRNA
tRNA
23S rRNA
5S rRNA
tRNA

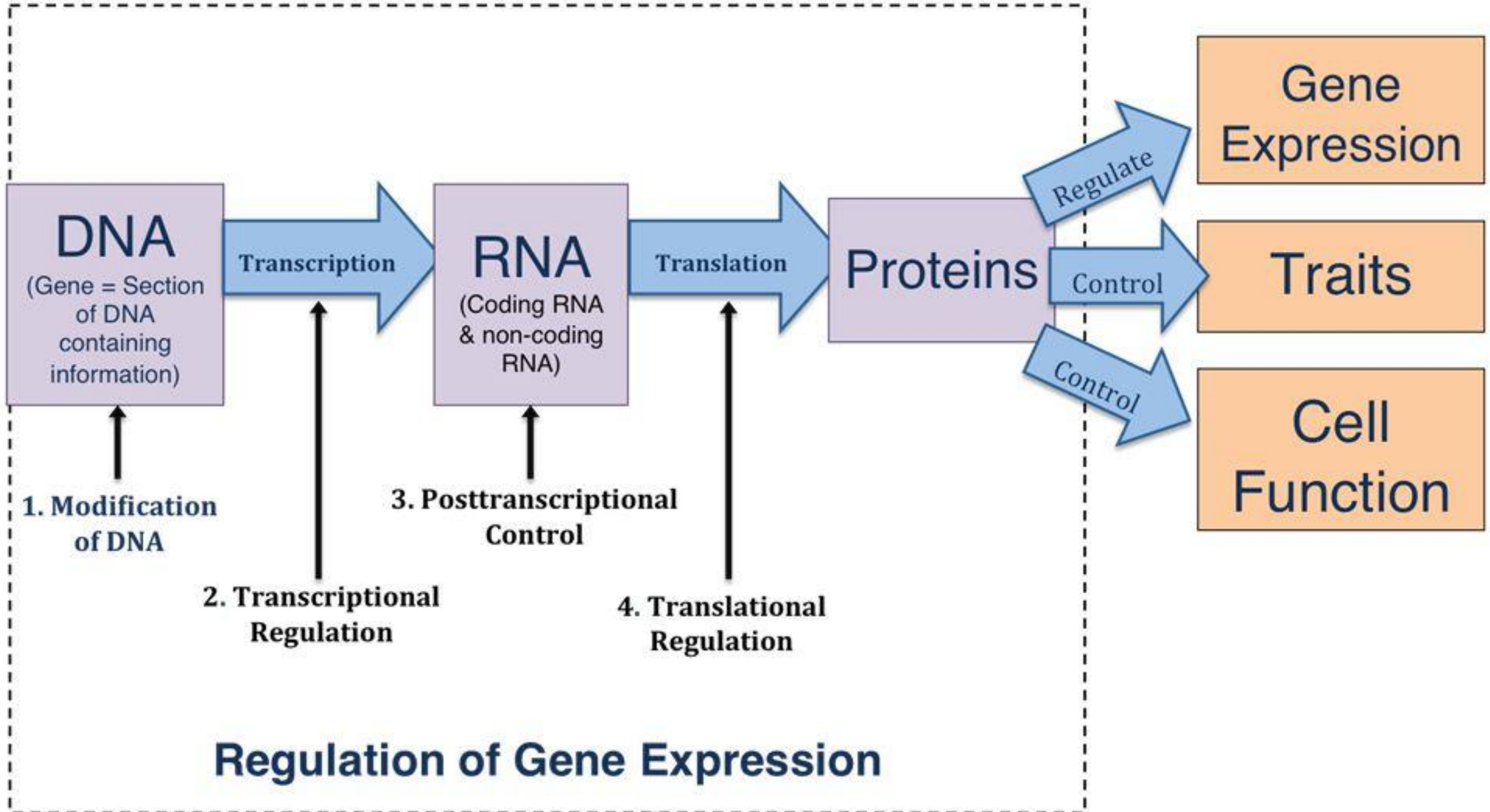
Composition of prokaryotic and eukaryotic chromosomes



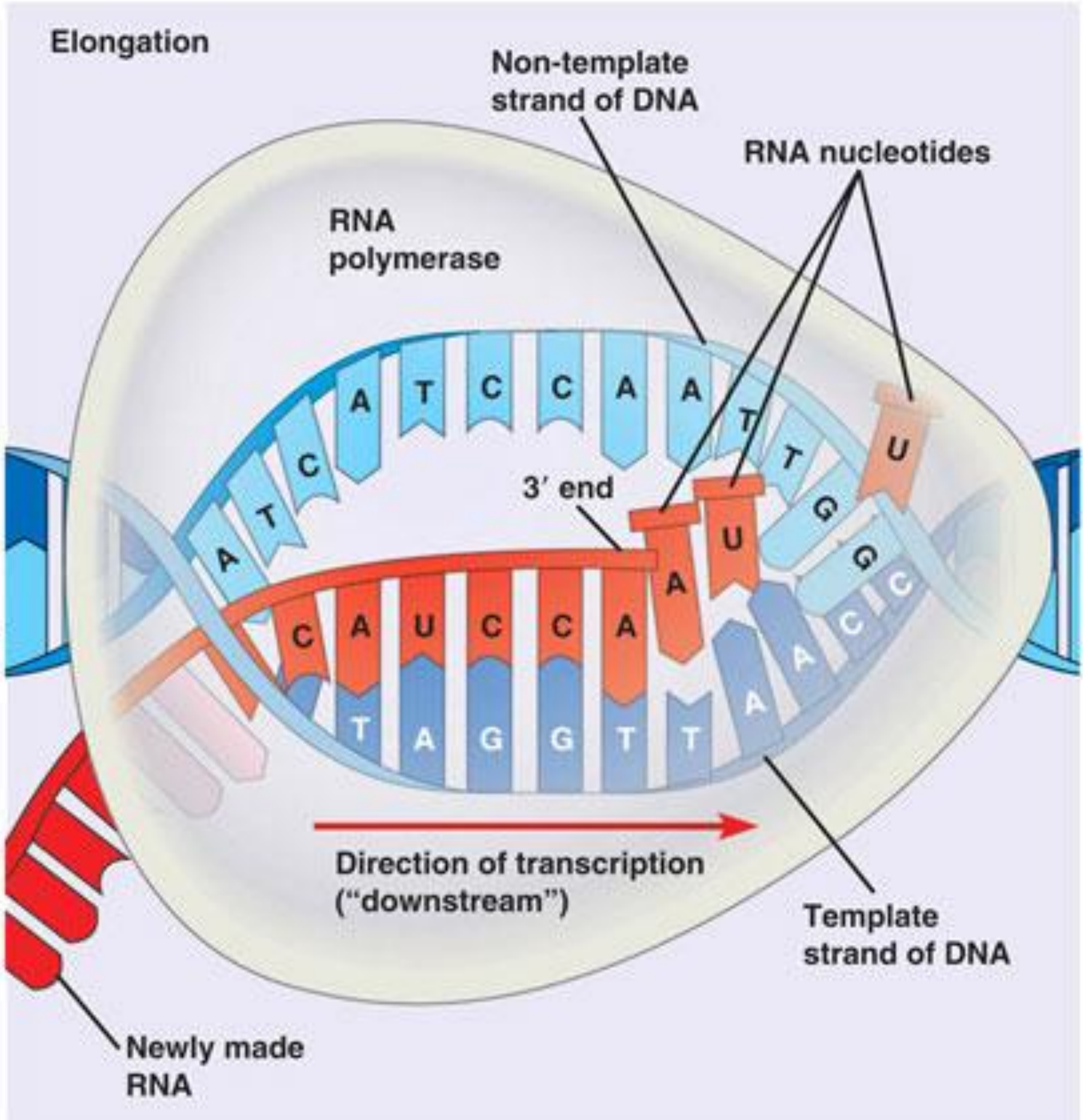
Although there are far more ribosomal proteins than rRNAs in each subunit, more than two-thirds of the mass of the prokaryotic ribosome is RNA. The ribosomal proteins are small (15 kD on average, while the 16 and 23S rRNAs are large (330 daltons per base, almost 1000 kD for 23S rRNA).

Fig.15.13

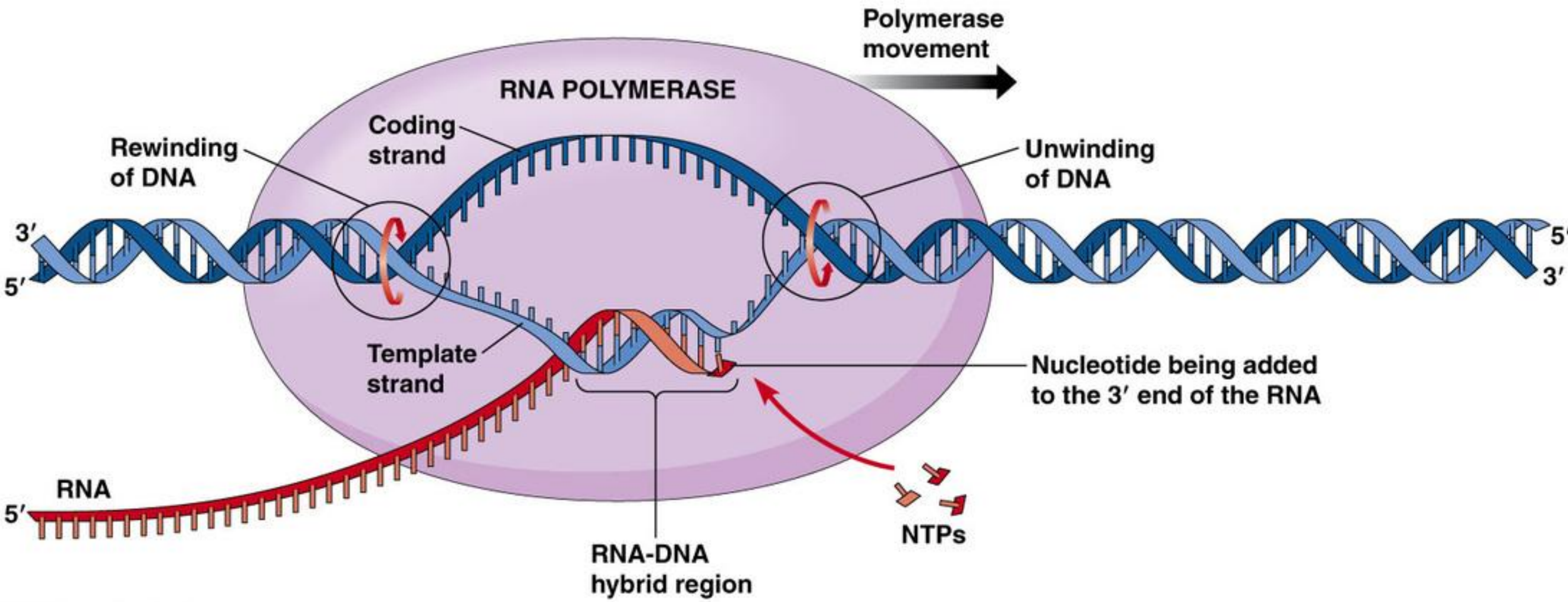
Gene Regulation

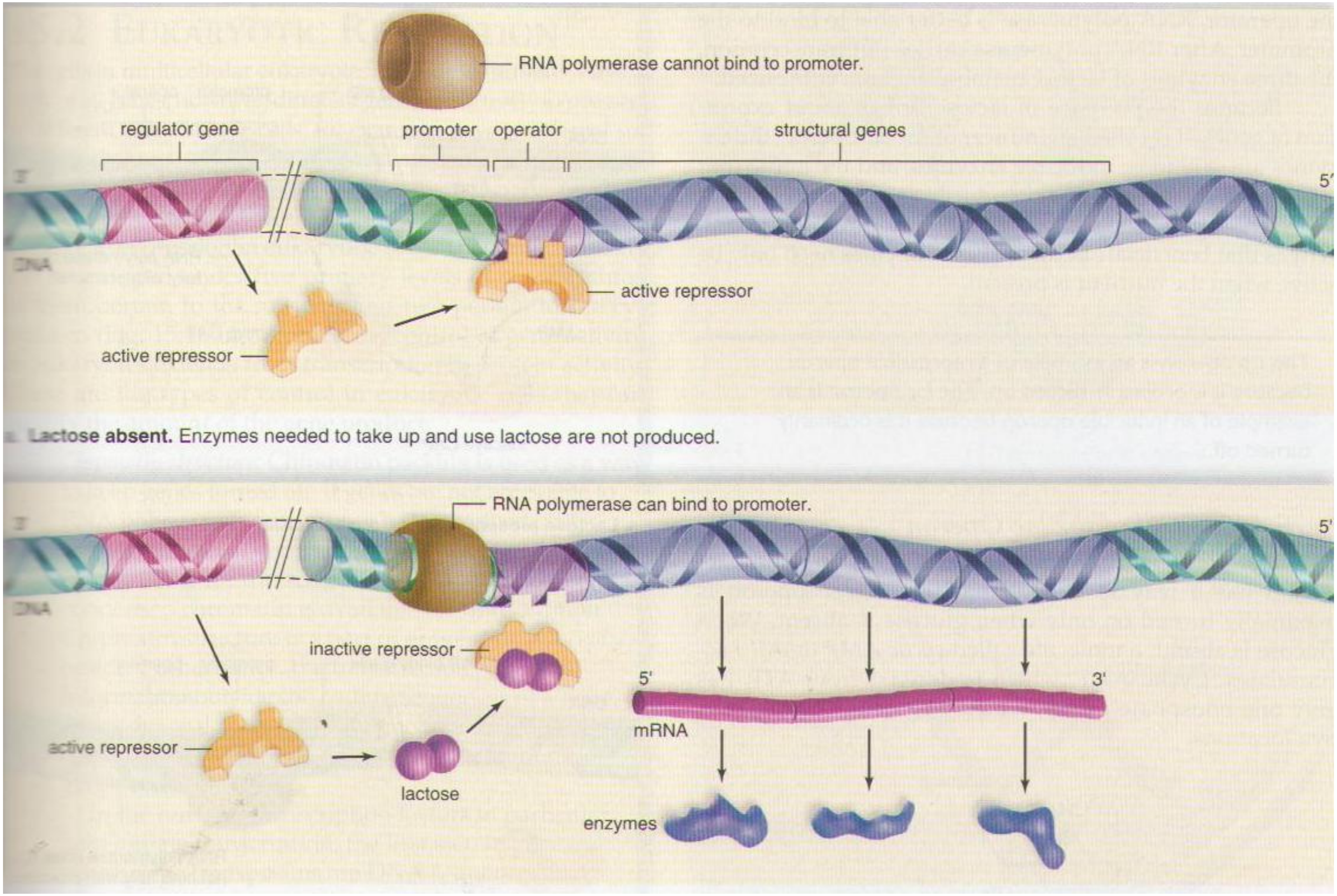


Graphic courtesy of Marianne Dobrovolny

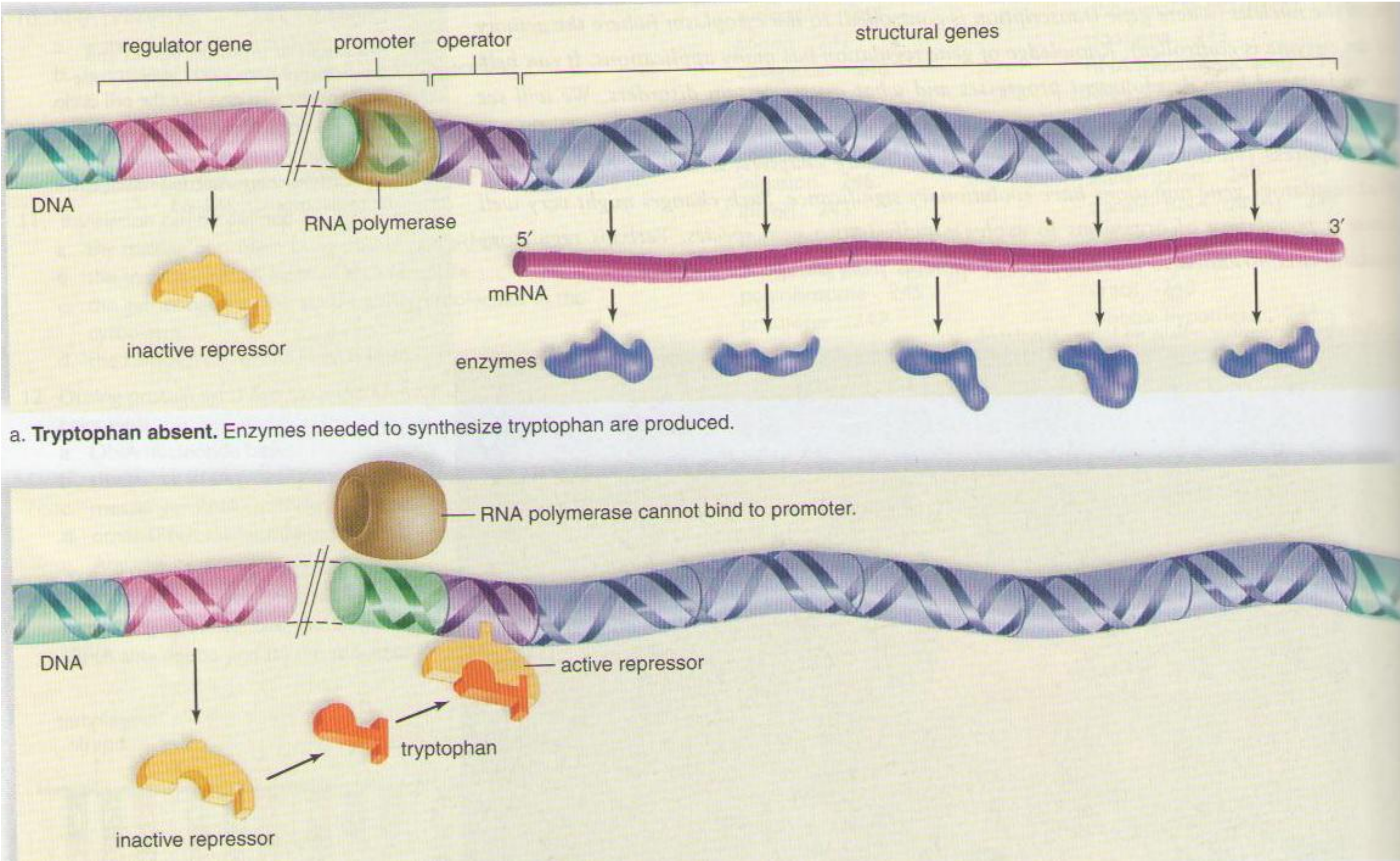


Elongation
Non-template strand of DNA
RNA nucleotides
RNA polymerase
3′
5′
5′
3′ end
A T C A T C C A A T T G G
C A U C C A A U U
T A G G T T A A C C
Direction of transcription ("downstream")
Template strand of DNA
Newly made RNA


Polymerase
movement
RNA POLYMERASE
Coding
strand
Rewinding
of DNA
Unwinding
of DNA
3′
5′
5′
3′
Template
strand
Nucleotide being added
to the 3′ end of the RNA
RNA
5′
NTPs
RNA-DNA
hybrid region



The *lac* operon (Inducible Operon)



The *trp* poeron (Repressible Operon)

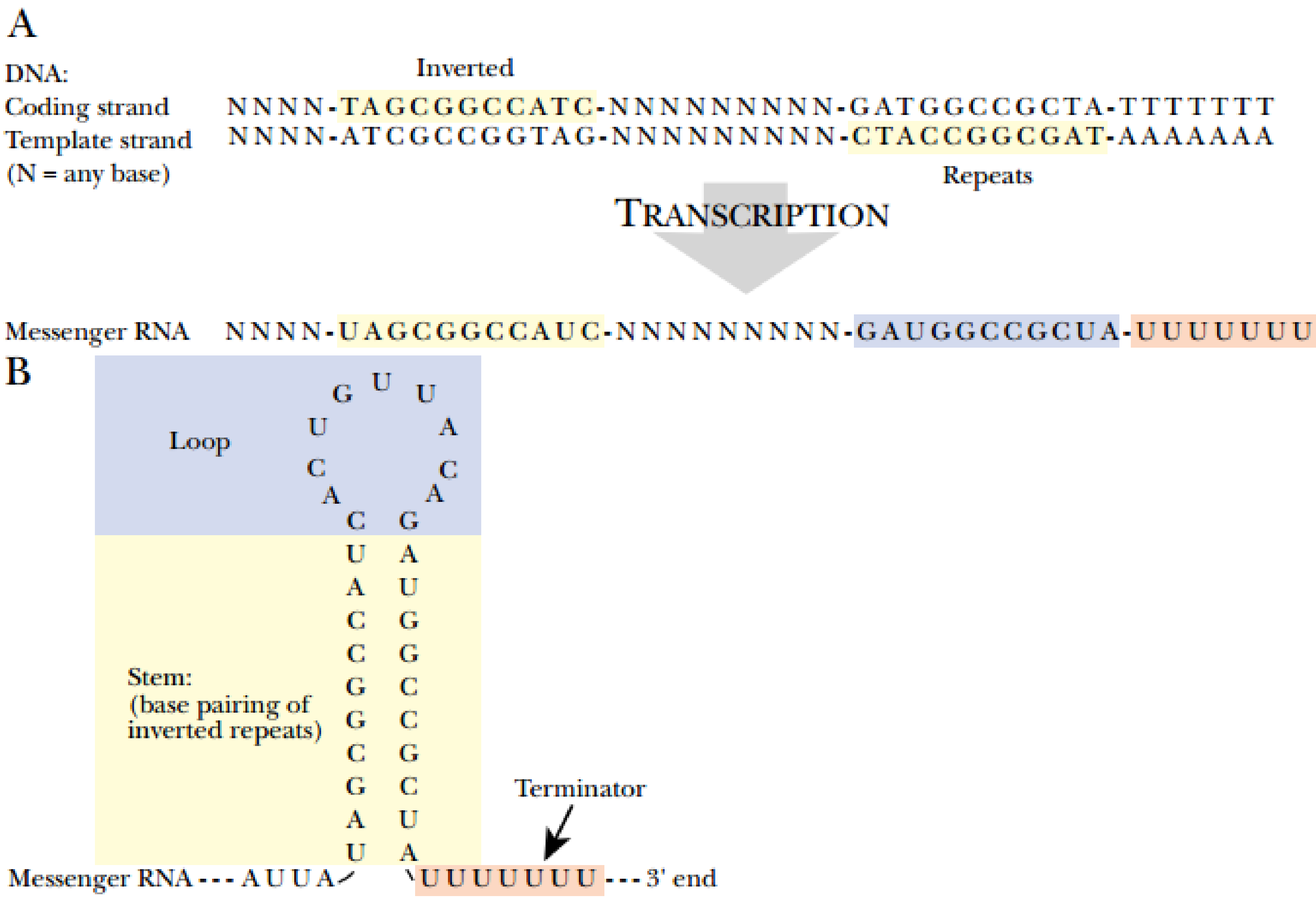
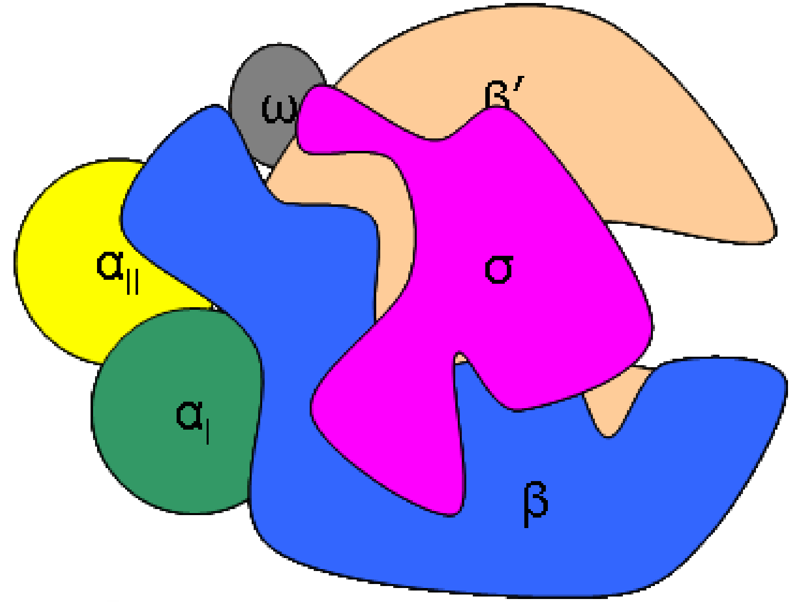
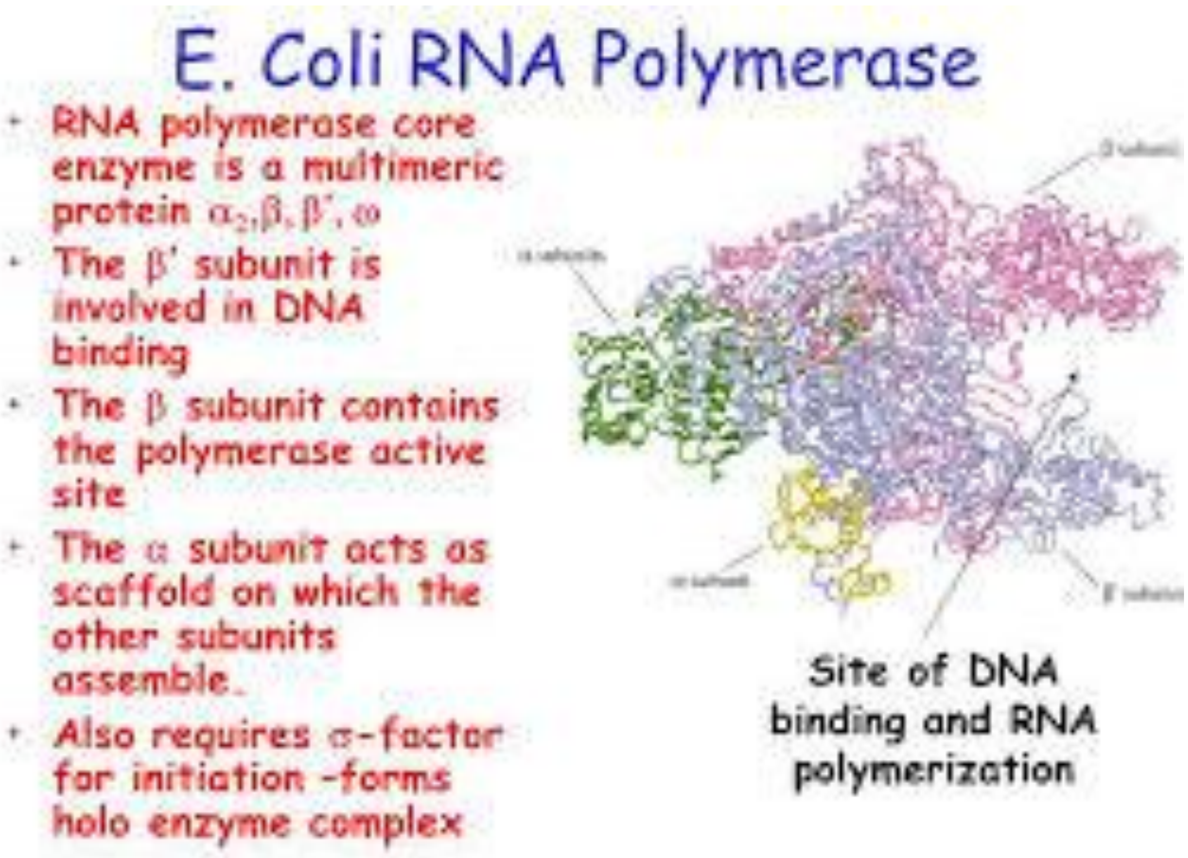


FIGURE 6.08 ***The Terminator Sequence is Transcribed into RNA***

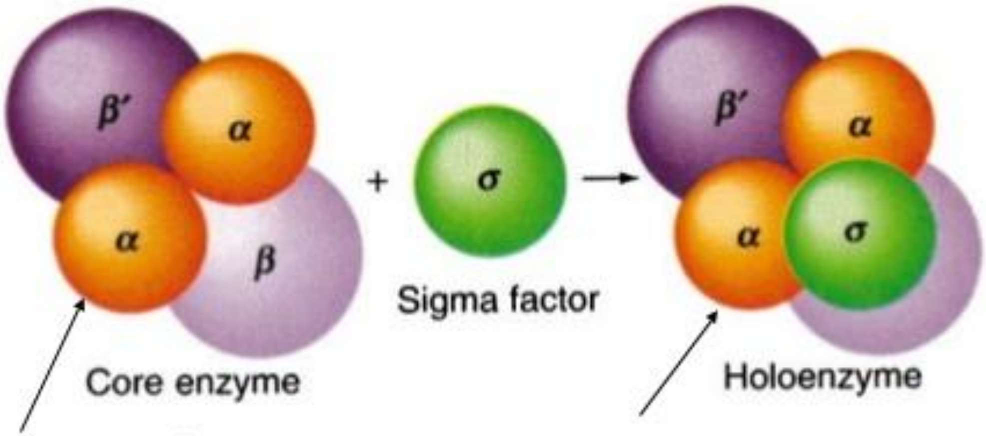
A) The signal for RNA polymerase to stop is shown in both the DNA and the RNA transcribed from it. The terminator consists of an inverted repeat separated by approximately 10 bases from a run of U's. B) The complementary bases form the stem of the hairpin, with the intervening bases forming the loop.


ω
β′
α_{II}
σ
α_I
β


E. Coli RNA Polymerase
RNA polymerase core enzyme is a multimeric protein $\alpha_2,\beta,\beta',\omega$
The β' subunit is involved in DNA binding
The β subunit contains the polymerase active site
The α subunit acts as scaffold on which the other subunits assemble.
Also requires σ-factor for initiation -forms holo enzyme complex
Site of DNA binding and RNA polymerization

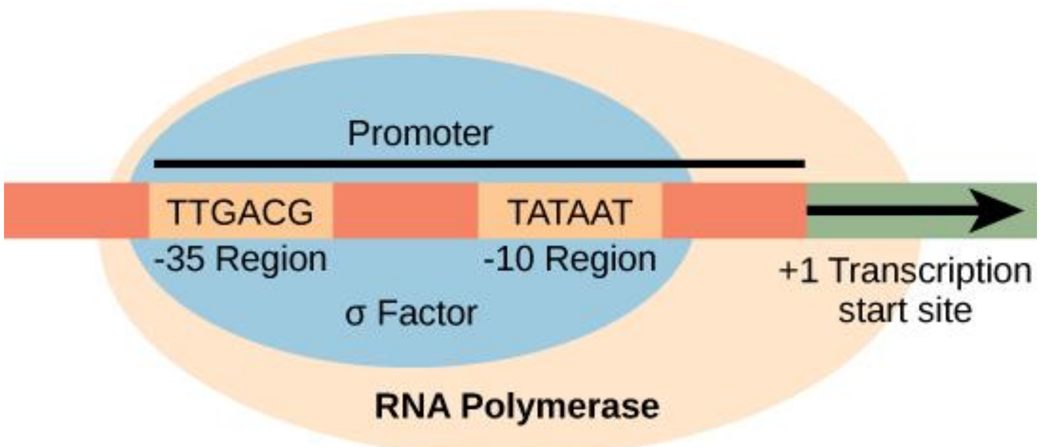
E. coli RNA polymerase

2α, 1β, 1β', 1ω and σ factor



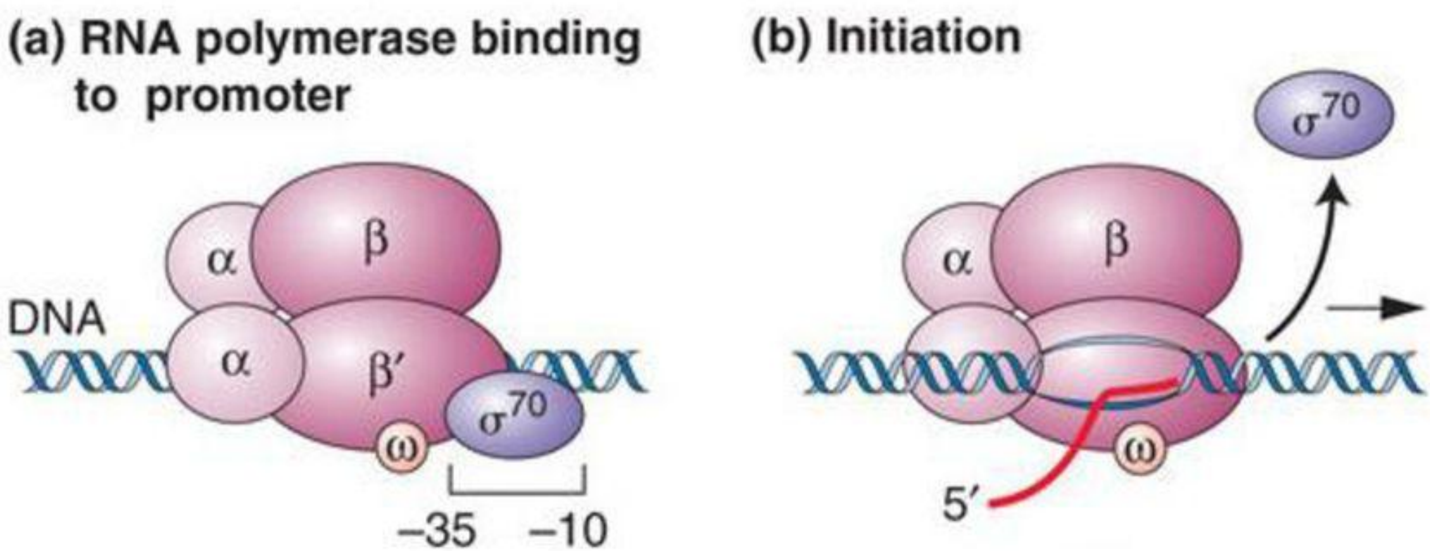
Required for polymerization activity

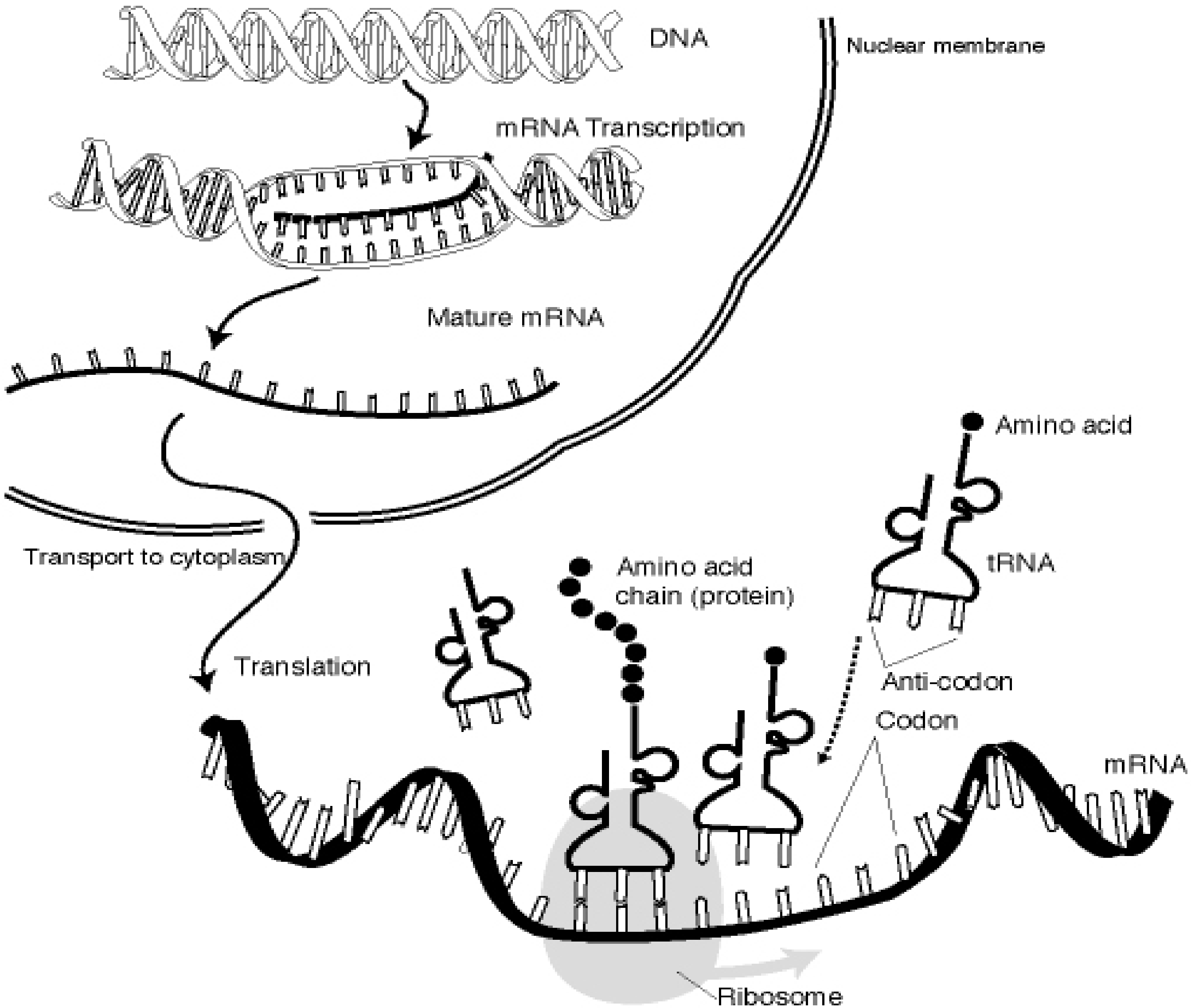
Required for correct initiation of transcription: ***binding to promoter***

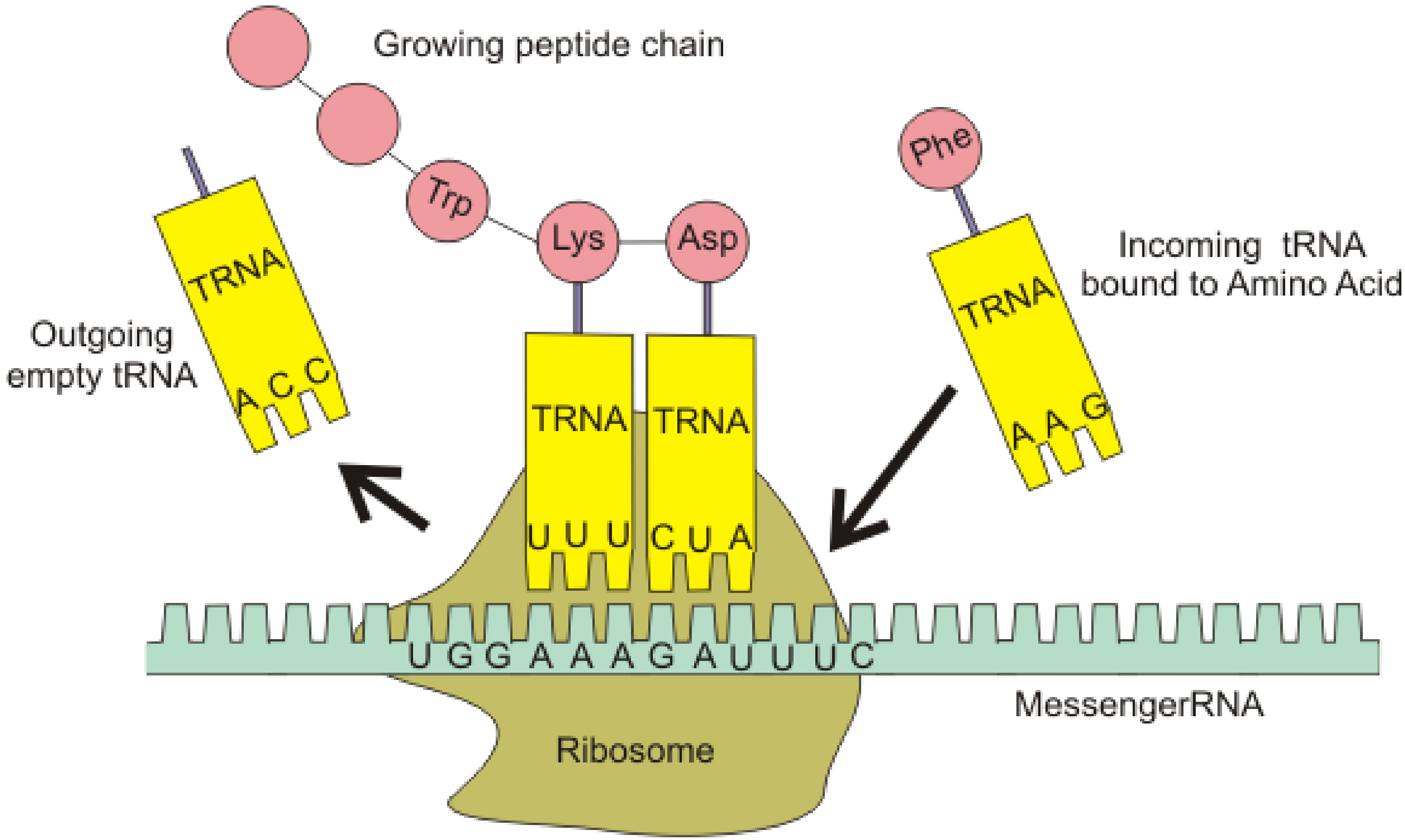


RNA polymerase holoenzyme

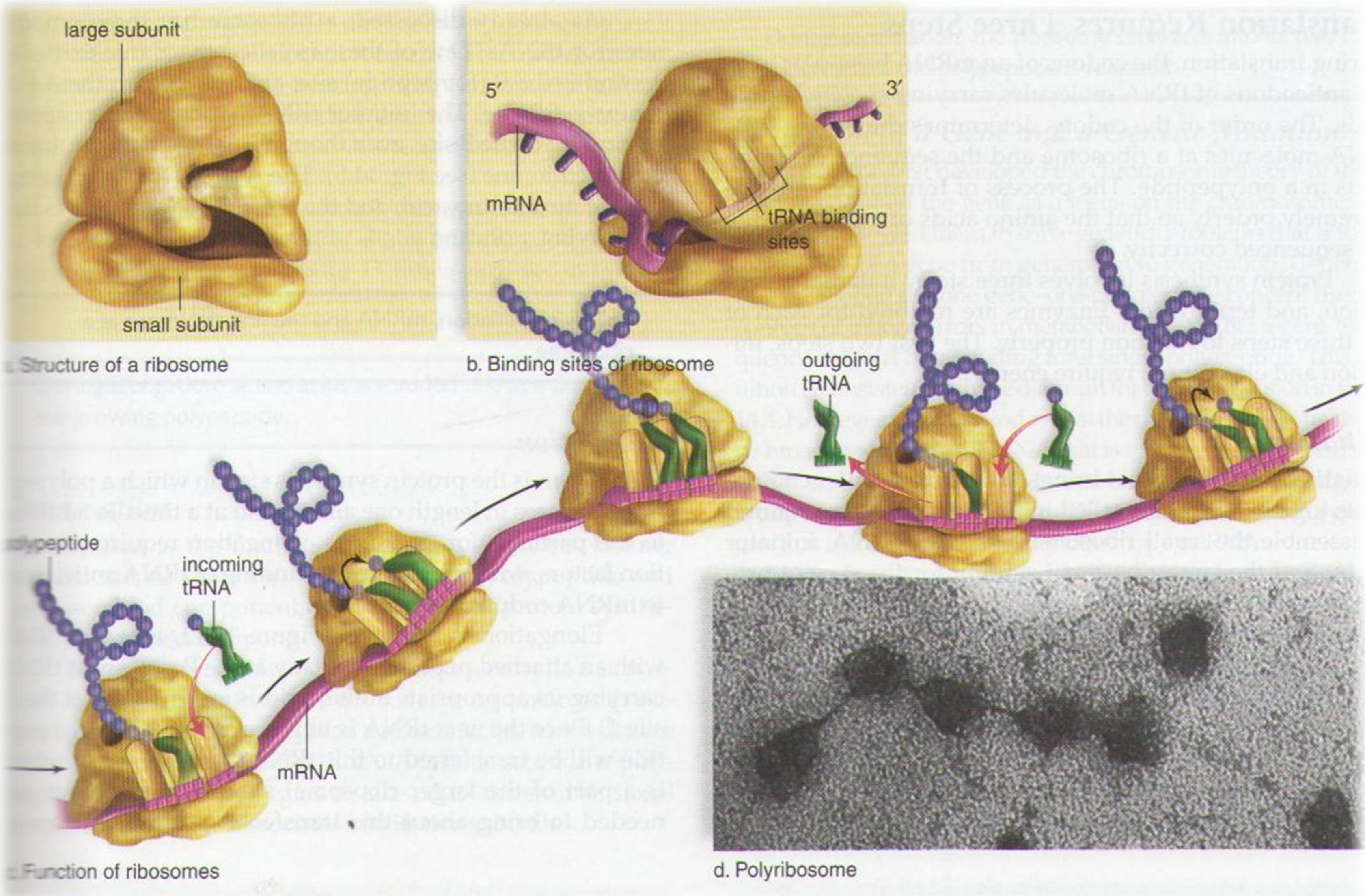
- Bacterial RNA polymerase complex that scans DNA for a promoter sequence (sigma factor binds)
- The **<u>RNA pol holoenzyme</u>** is a multi-subunit complex



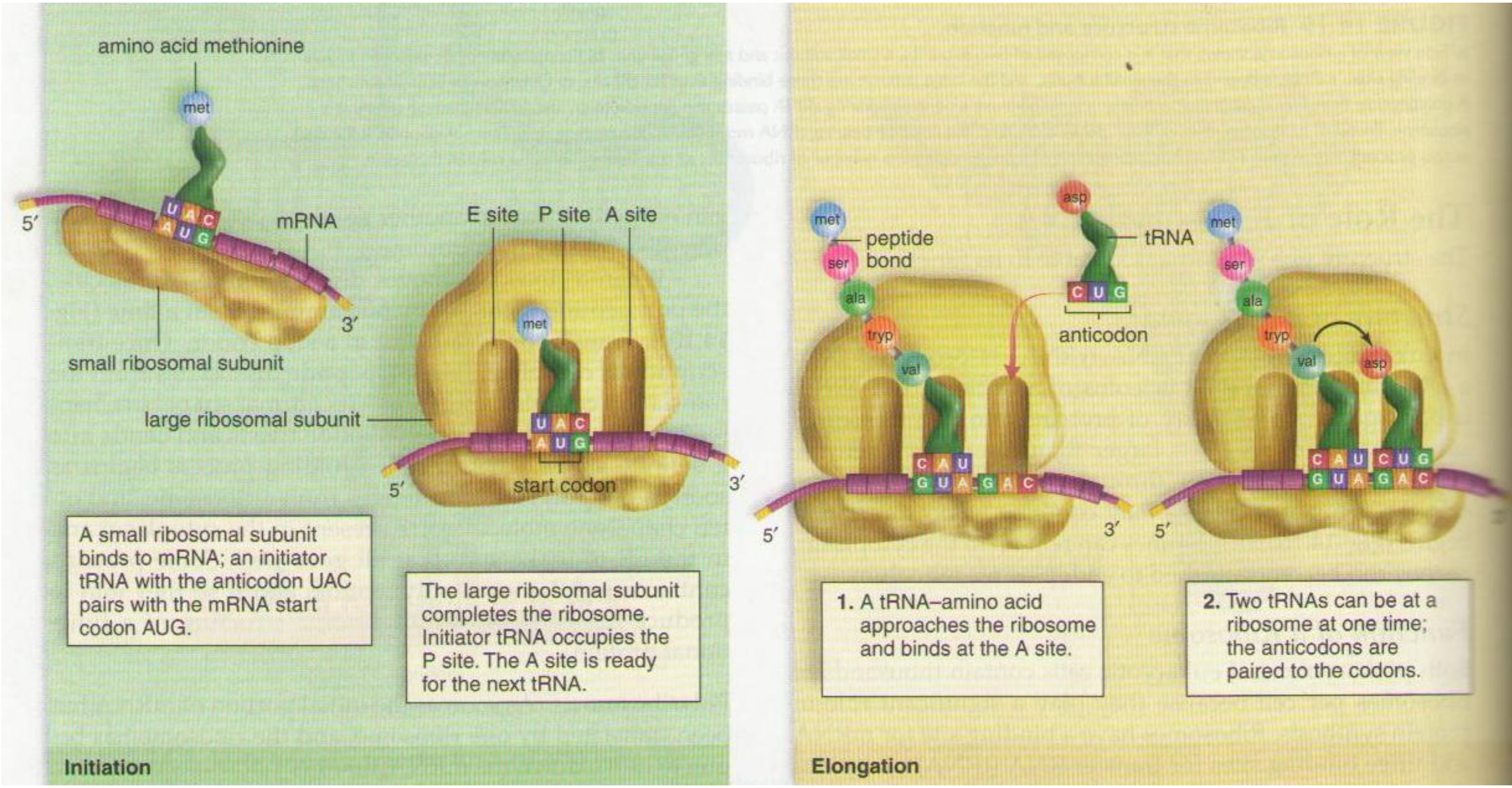

DNA
Nuclear membrane
mRNA Transcription
Mature mRNA
Amino acid
Transport to cytoplasm
Amino acid
chain (protein)
tRNA
Translation
Anti-codon
Codon
mRNA
Ribosome

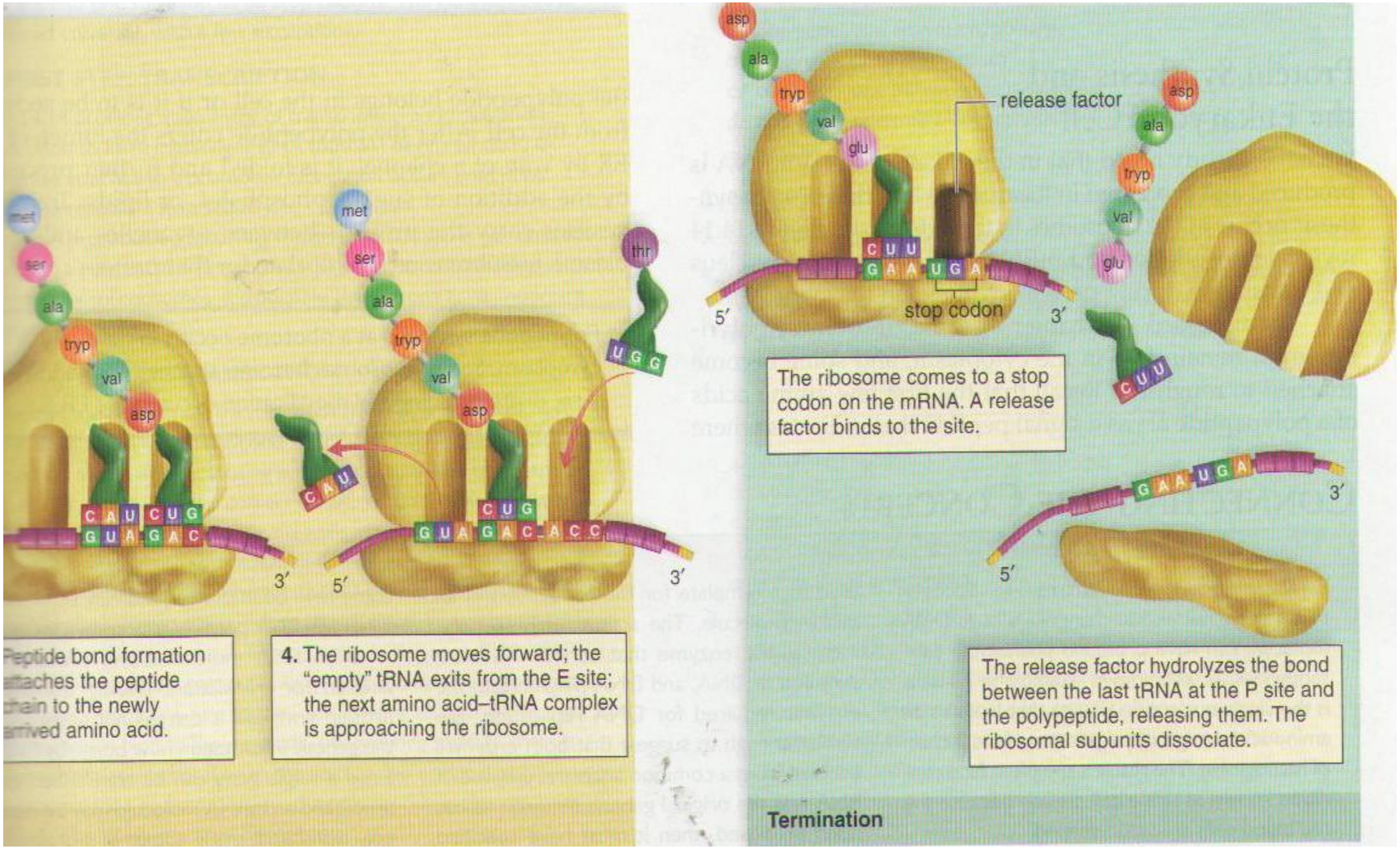


Peptide Synthesis



a. Structure of a ribosome
b. Binding sites of ribosome
c. Function of ribosomes
d. Polyribosome


amino acid methionine
met
U A C
A U G
5′
mRNA
3′
small ribosomal subunit
E site
P site
A site
large ribosomal subunit
start codon
A small ribosomal subunit binds to mRNA; an initiator tRNA with the anticodon UAC pairs with the mRNA start codon AUG.
The large ribosomal subunit completes the ribosome. Initiator tRNA occupies the P site. The A site is ready for the next tRNA.
Initiation
peptide bond
met
ser
ala
tryp
val
asp
tRNA
C U G
anticodon
C A U
G U A G A C
1. A tRNA–amino acid approaches the ribosome and binds at the A site.
2. Two tRNAs can be at a ribosome at one time; the anticodons are paired to the codons.
Elongation



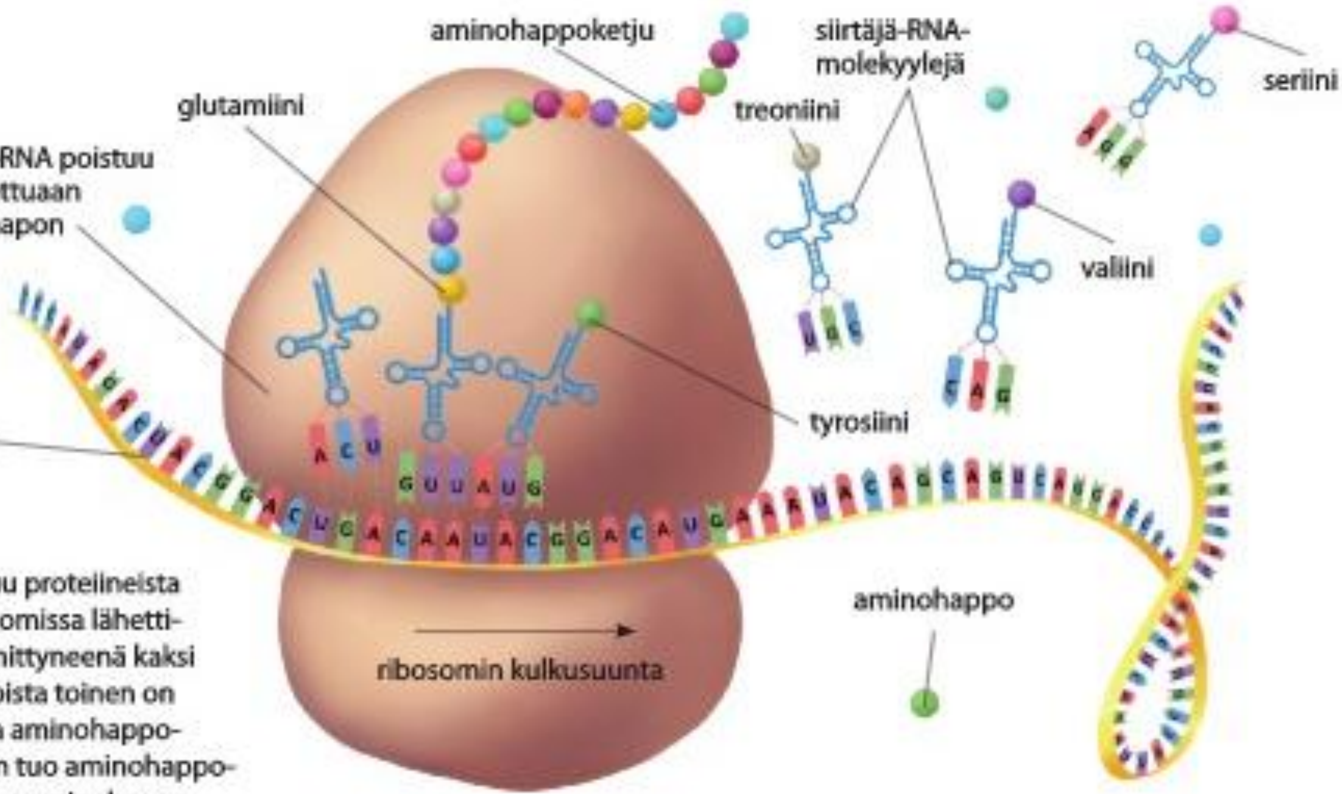
Peptide bond formation attaches the peptide chain to the newly arrived amino acid.

4. The ribosome moves forward; the "empty" tRNA exits from the E site; the next amino acid–tRNA complex is approaching the ribosome.

The ribosome comes to a stop codon on the mRNA. A release factor binds to the site.

The release factor hydrolyzes the bond between the last tRNA at the P site and the polypeptide, releasing them. The ribosomal subunits dissociate.

Translaatio



Ribosomi koostuu proteiineista ja RNA:sta. Ribosomissa lähetti-RNA:han on kiinnittyneenä kaksi siirtäjä-RNA:ta, joista toinen on kiinni kasvavassa aminohappoketjussa ja toinen tuo aminohappoketjuun seuraavan aminohapon. Aminohappojen välille muodostuu peptidisidos. Tämän jälkeen ribosomi siirtyy yhden emäskolmikon verran eteenpäin ja liittää taas ketjuun uuden aminohapon.

FIGURE 7.15 ***Two Domains in Arabinose Binding Protein***

The arabinose binding protein of *E. coli* contains two open twisted α/β domains of similar structure. A) Schematic diagram of a single domain. B) Topology diagram showing the orientation of the two domains and the crevice between them in which the arabinose molecule binds. From: Introduction to Protein Structure by Brandon & Tooze, 2nd ed., 1999. Garland Publishing, Inc., New York and London.