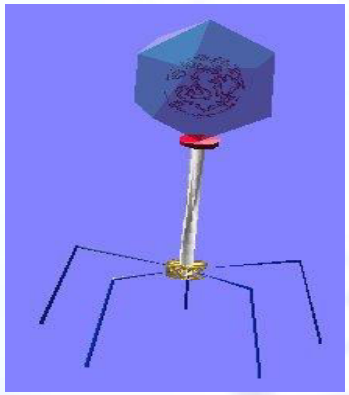
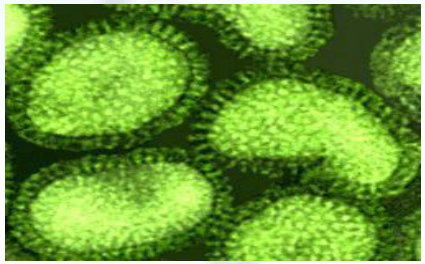
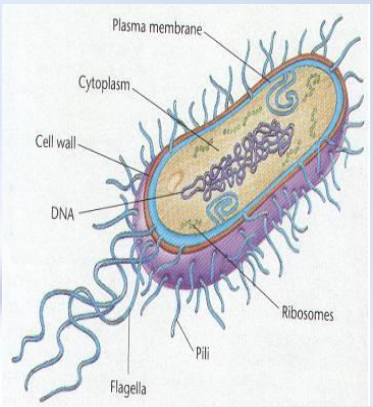






General Microbiology

Lec 7

Microbial Genetic

Ammar Algburi

Ph.D. in Microbial Biology

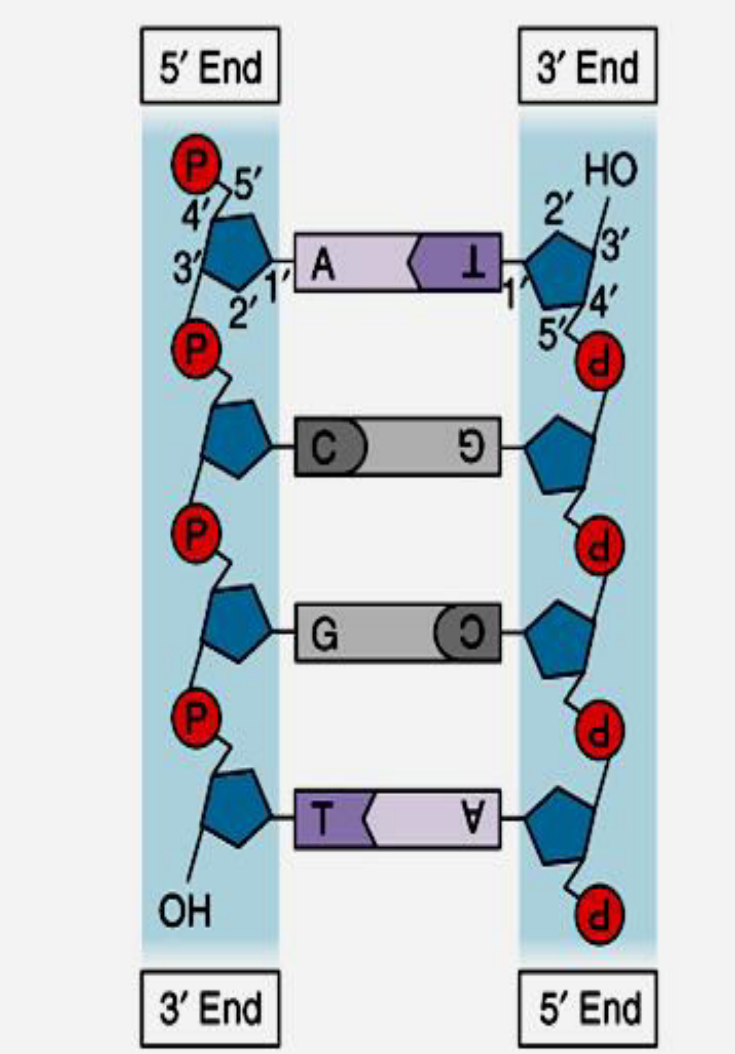
Nucleic Acids

- Composed of chains of nucleotides (several thousands or millions of nucleotides)
- Usually composed of 4 different nucleotides
- Each nucleic acid molecule has its own order, or "sequence," of nucleotides
- The correct sequence of nucleotides is essential for the nucleic acid's function

Nucleotide structure

- A nucleotide consists of:
  - Nitrogenous base
  - Pentose sugar
  - Phosphate group
- Nitrogenous bases:
  - Purines: adenine & guanine
  - Pyrimidines: cytosine, thymine (in DNA), & uracil (in RNA)
- Pentose sugars:
  - Ribose (found in RNA)
  - Deoxyribose (found in DNA)

DNA: Deoxyribonucleic acid



- Pentose sugar: 2’-deoxyribose
- Nitrogenous bases:
  Adenine and Guanine (purines)
  Cytosine and Thymine (pyrimidines)
- Structure is typically a double-stranded helix
- 5’ to 3’ (strands are anti-parallel)
- Nucleotide sequences of the strands are complementary to each other, A =T and C = G

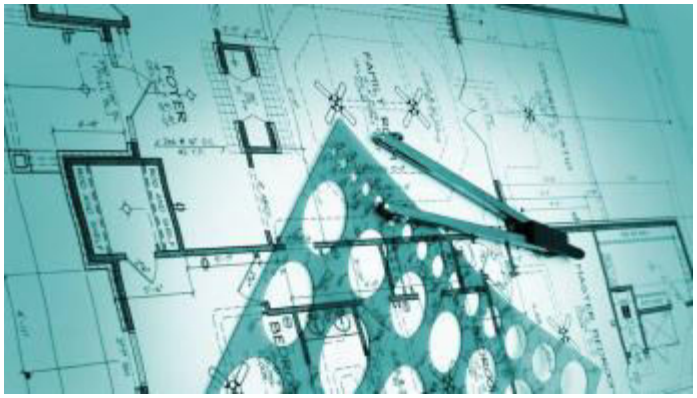
RNA: Ribonucleic acid

- Pentose sugar: Ribose
- Nitrogenous bases:
  Adenine and Guanine (purines)
  Cytosine and Uracil (pyrimidines)
- Structure is typically single-stranded (C to G; A to U)
- An RNA strand can also form a double-stranded structure with a DNA strand; in this case, the U on the RNA = A on the DNA.

Types of RNA

Genetic information copied from DNA is transferred to 3 types of RNA:

- **messenger** (mRNA) **is like a**
  Copy of information in DNA that is brought to the ribosome where the information is translated into a protein.



- **ribosomal** (rRNA) **is like a**
  The protein factories of the cells.

- **transfer** (tRNA) **are like a**
  Brings the amino acid to the ribosome.

Genetic Code

- rRNA: triplet code
- mRNA: codon (complimentary to triplet code of DNA)
- tRNA: anticodon (complimentary to codon)

Genetic Code

- Codons: code for the production of a specific amino acid
- 20 amino acids
- 3 base code
- Degenerative: more than 1 codon codes for an amino acid
- Universal: in all living organisms

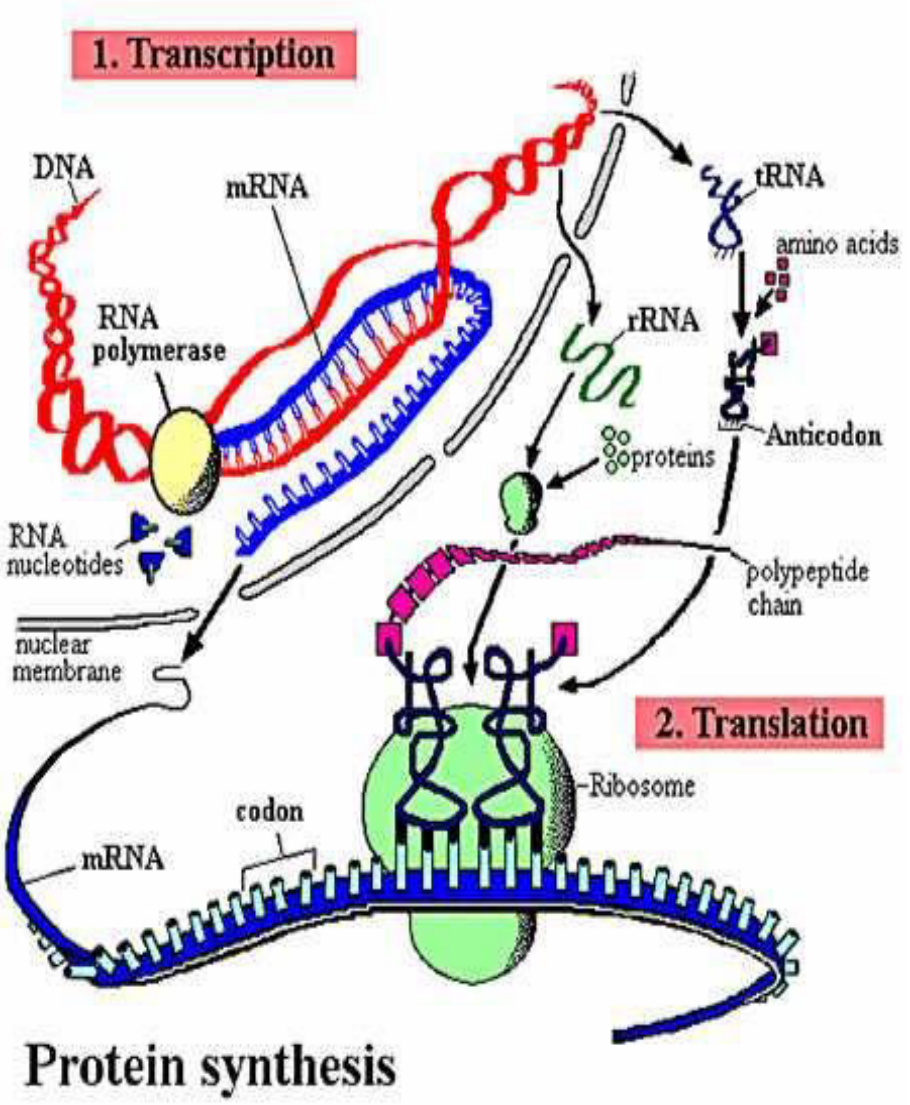
Overall function.

- The nucleotide sequence of a nucleic acid molecule encodes the amino acid sequence of a protein.
- Genome: The entire nucleotide sequence of an organism; transmitted to offspring during reproduction
- Deoxyribonucleic acid (DNA): DNA molecules serve as the genome for the proteins of all cellular organisms, both eukaryotic and prokaryotic. DNA also serves as the genome for certain viral groups.
- Ribonucleic acid (RNA): RNA molecules serve as an intermediate in gene expression in eukaryotic and proyaryotic organisms, as well as some viruses. RNA serves as the genome for certain viral groups.

Nucleic Acid Function

Gene Expression:

[Transcription & Translation]



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DNA Replication

- Bacteria have closed, circular DNA
- Genome: genetic material in an organism
- *E. coli*
  - 4 million base pairs
  - 1 mm long (over 1000 times larger that actual bacterial cell)
  - DNA takes up around 10% of cell volume

DNA Replication-occurs at the replication fork

- 5’ to 3 ‘
- DNA helicase-unzips + parental DNA strand that is used as a template
  - Leading stand (5’ to 3’-continuous)

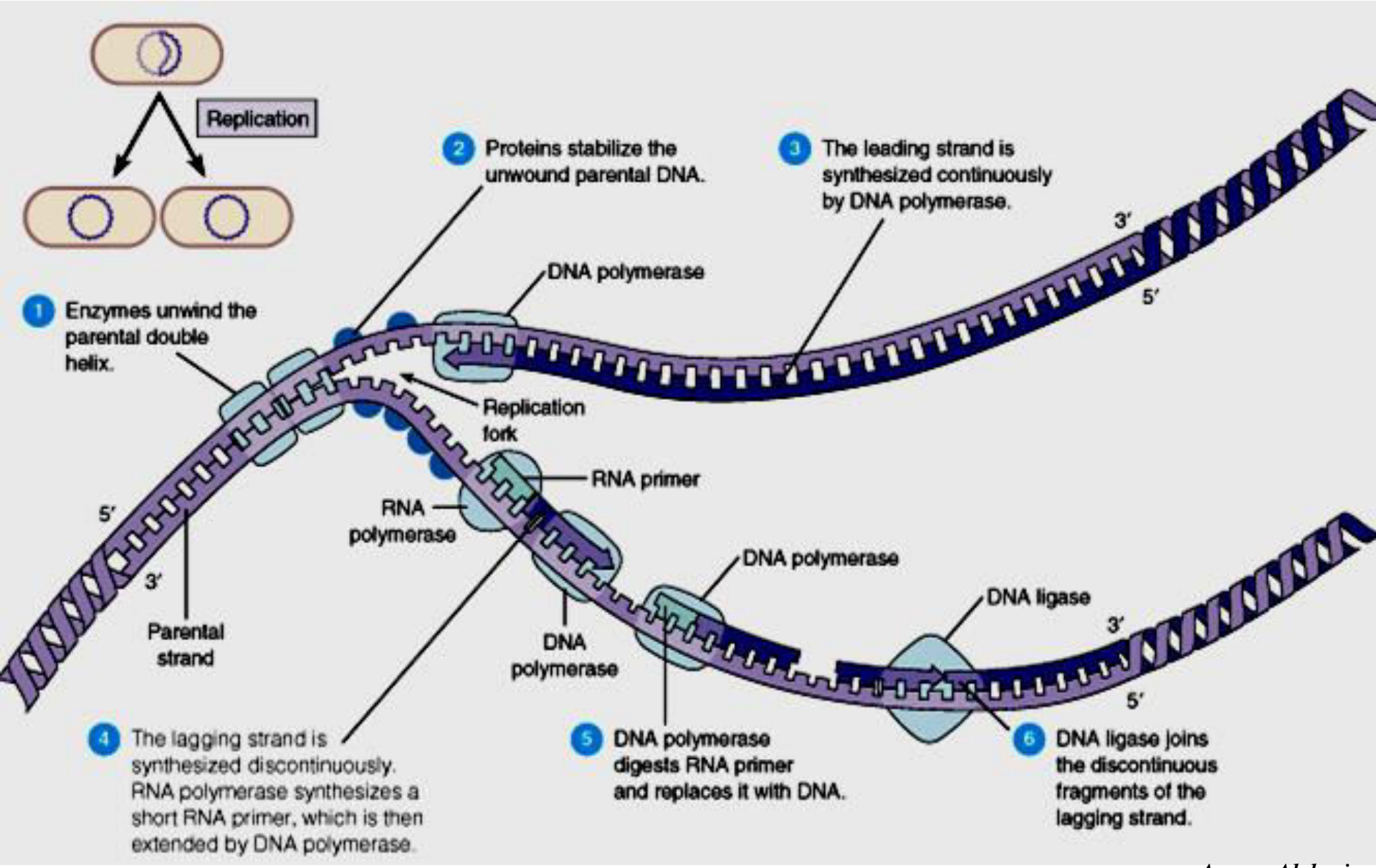
    *DNA polymerase-joins growing DNA strand after nucleotides are aligned (complimentary)
  - Lagging strand (5’ to 3’-not continuous)

    *RNA polymerase (makes short RNA primer)

    *DNA polymerase (extends RNA primer then digests RNA primer and replaces it with DNA)

    *DNA ligase (seals Okazaki fragments-the newly formed DNA fragments)

DNA Replication in Detail:



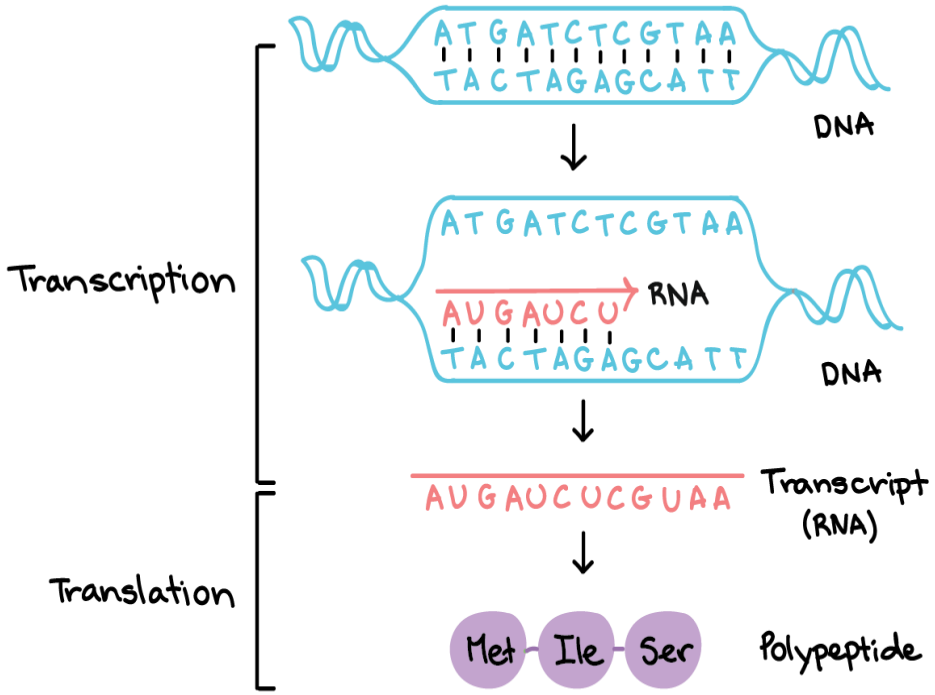
Protein Synthesis

- DNA-------→ mRNA------→ protein

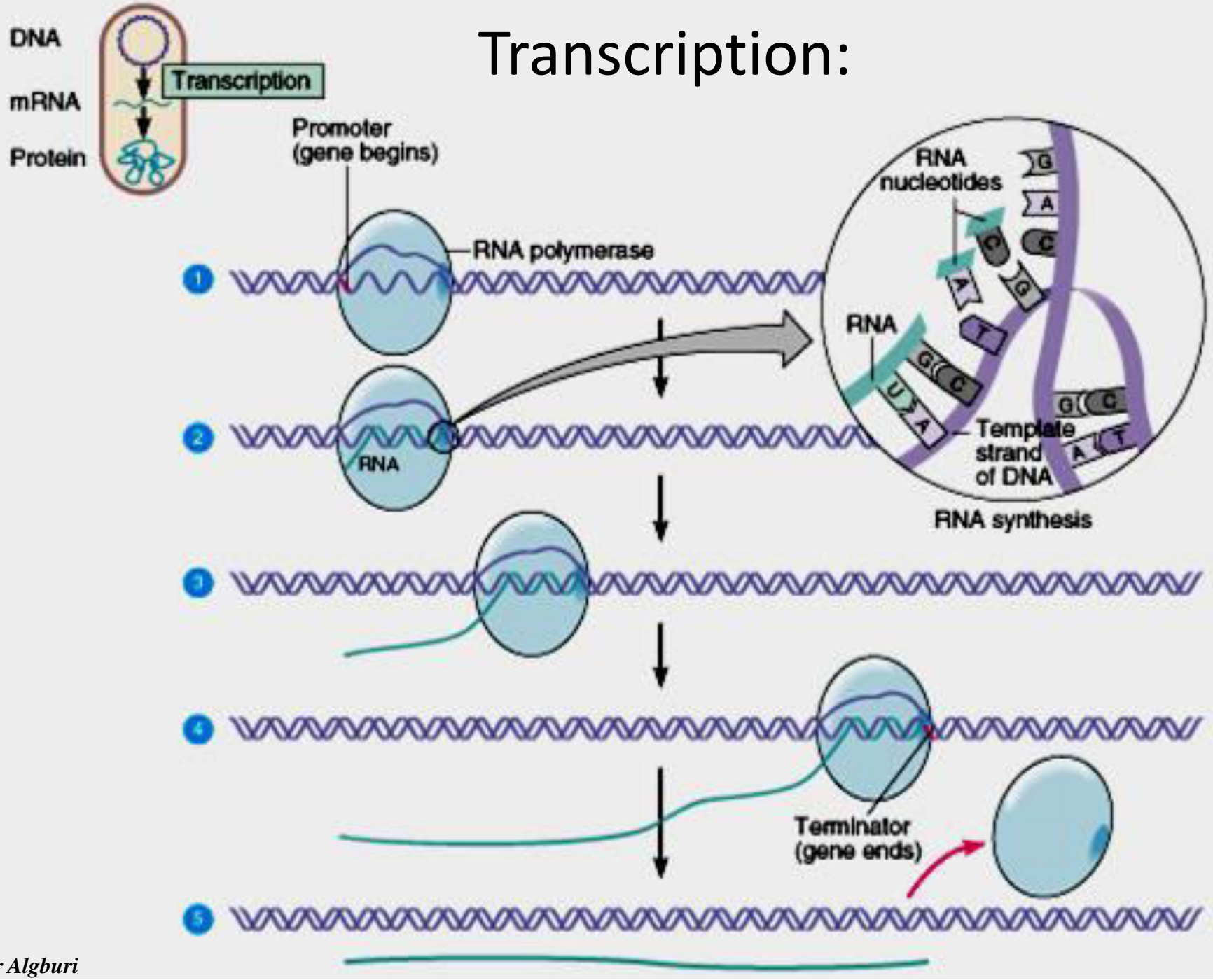
transcription translation

Central Dogma
of Molecular Genetics

- **Transcription**: The sequence of nucleotides in a DNA molecule serves as a template for the synthesis of an RNA molecule; typically, only a small segment of the DNA is copied. This is the first step in gene expression.



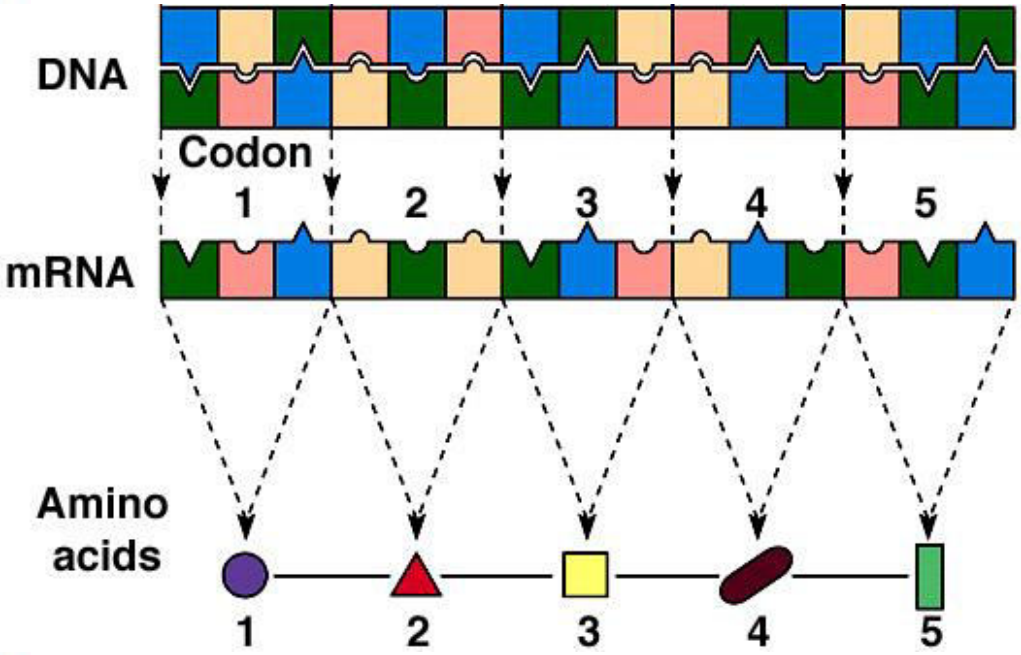
Transcription:



The Genetic Code

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

- **Every three mRNA nucleotides represent a codon to be translated into a particular amino acid based on the Genetic Code.**
- **Translation begins at the Start Codon (AUG) and proceeds codon by codon there after until a Stop Codon (one of three) is reached.**



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Translation (Protein Synthesis):

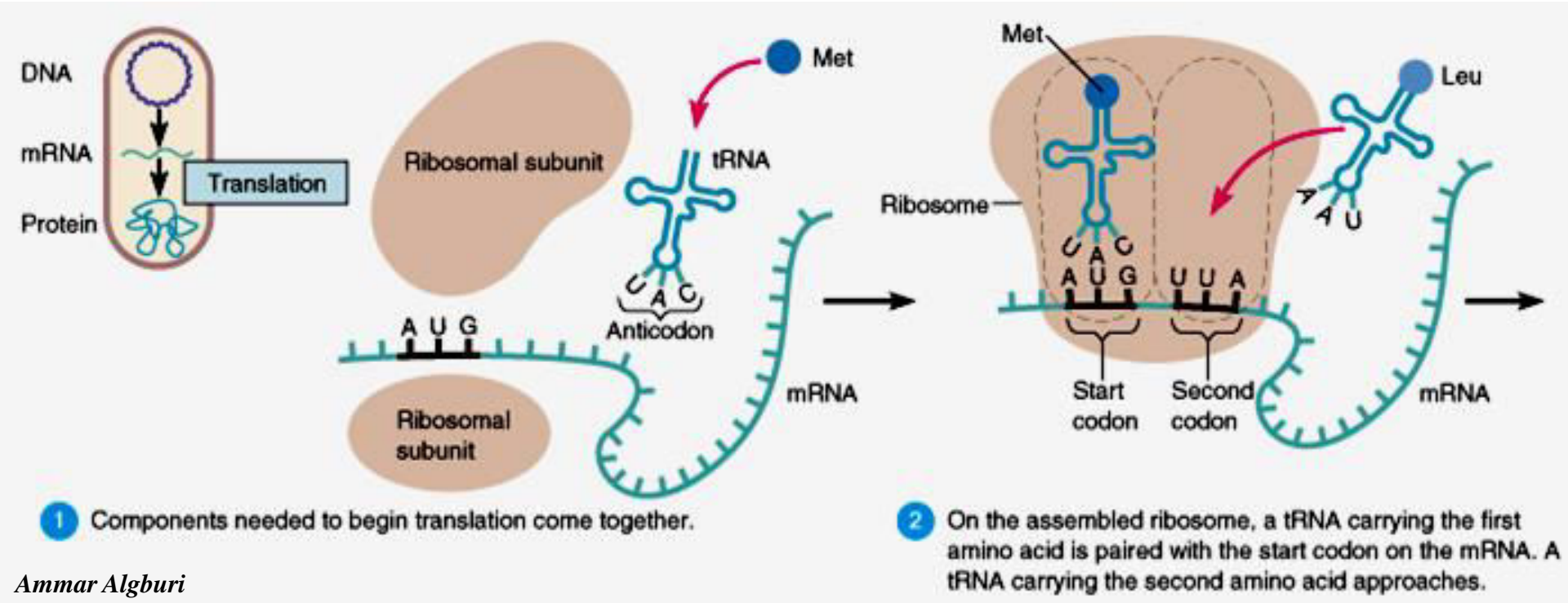
- **Translation**: The sequence of nucleotides in an RNA molecule serves to direct the assembly of amino acids into a protein chain on a ribosome. This is the second step in gene expression.

Translation (Protein Synthesis):

- **Codons** and anticodons, on tRNA.

- **Start codon** is first recognized by ribosome subunits, which sandwiches the mRNA and allows the first tRNA to bind by base pair complementation between mRNA codon and tRNA anticodon.

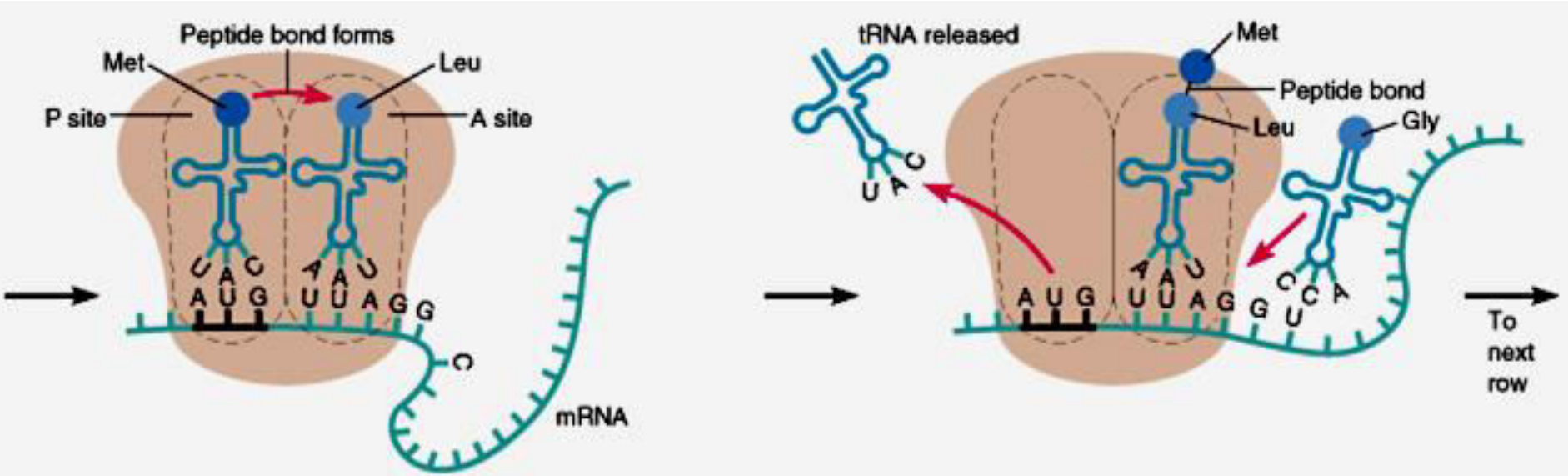
- **tRNAs only bind if "charged" with its correct amino acid (methionine) for start codon).**



• The ribosome moves down the mRNA by one codon (3 nucleotides) to make room for addition of the next charged tRNA in what is called the ribosome A-site.

• Once a second charged tRNA binds at the A-site, a peptide bond will form between the amino acid in the P-site and that on the tRNA in the A-site.

• The "uncharged" tRNA will release from the P-site and, again, the ribosome will move down the mRNA by one codon.



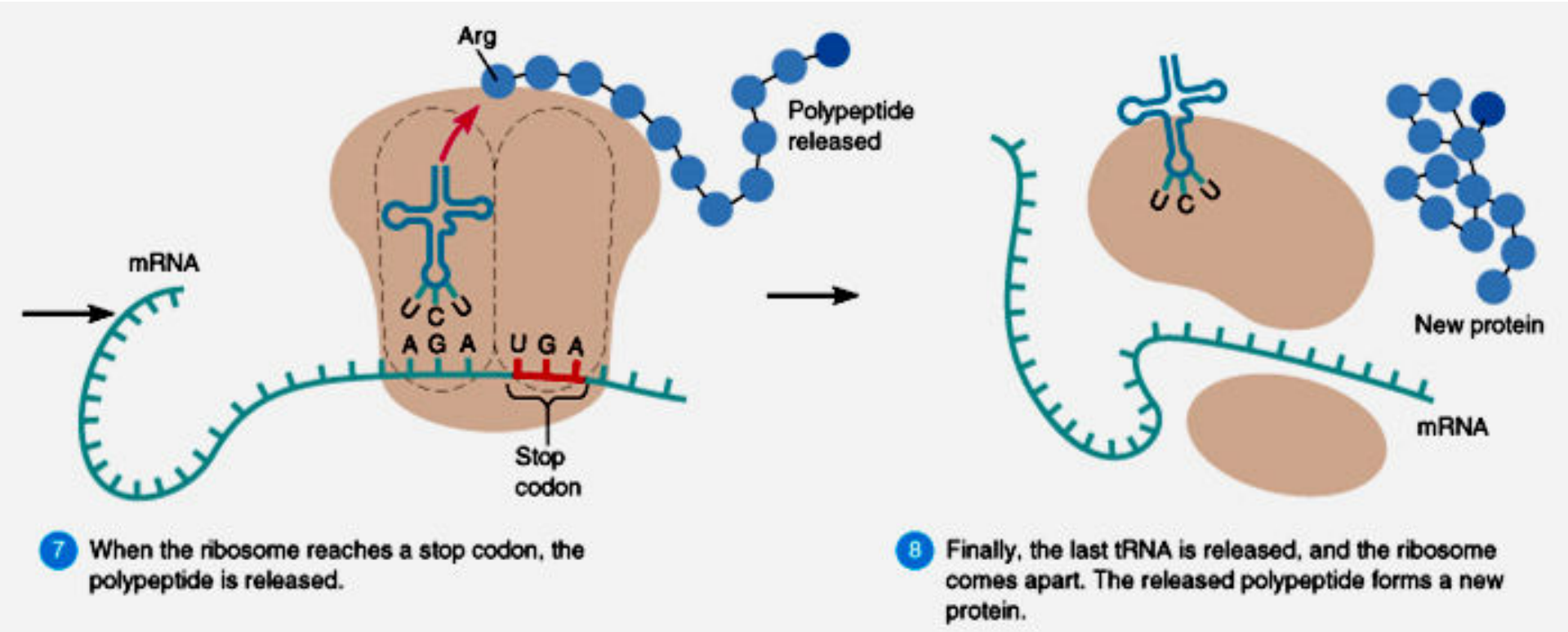
3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.

4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

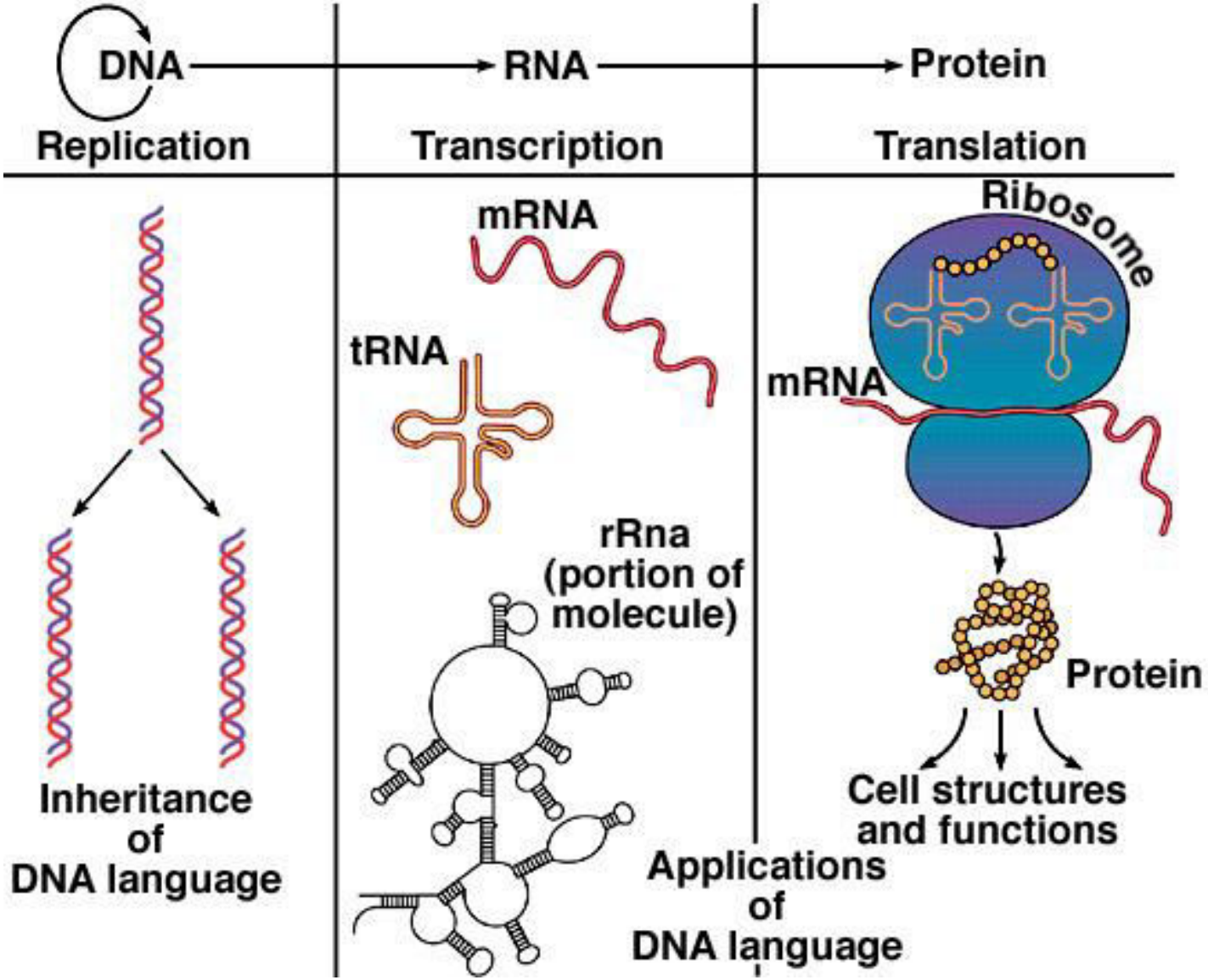
• The process continues, codon by codon, adding amino acids to a growing polypeptide, until the ribosome reaches a Stop Codon.

• No new amino acid is added by the stop codon, rather it facilitates the release of the last amino acid from its tRNA; thereby releasing the polypeptide.

• Again the polypeptide may now fold into a functional protein.



The Central Dogma of Biology



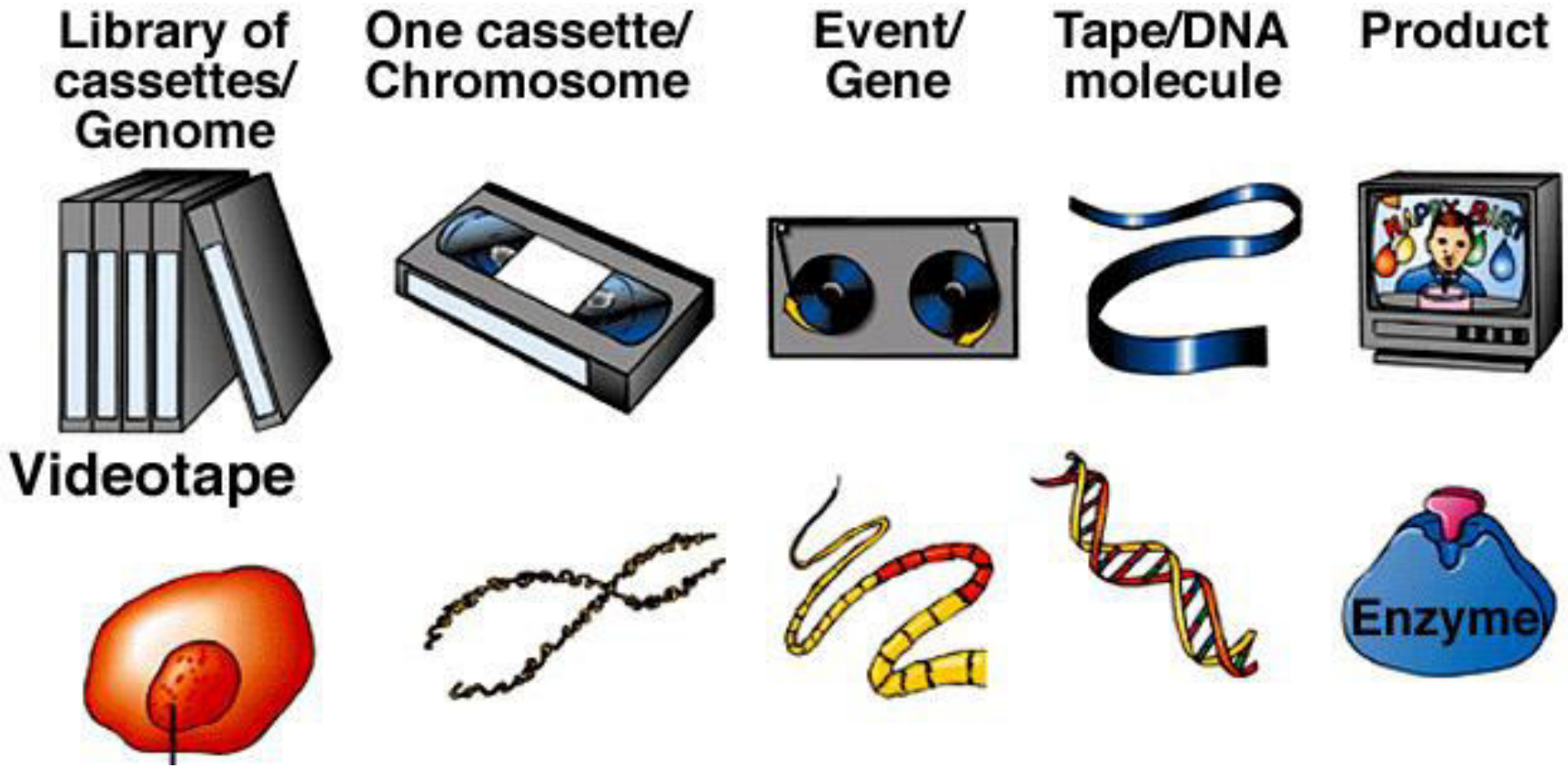
- Genes play three notable roles:
  - To encode the nucleotide sequences of mRNA, which in turn encodes the amino acid sequences of proteins
  - To encode the nucleotide sequences of tRNA or rRNA
  - To regulate the expression of other genes
- Mutation:
  - Change in the nucleotide sequence of a gene, usually resulting from an error during DNA replcation

Geno- and pheno- type

- Phenotype
  - The appearance or discernible characteristics of an individual
  - Mutation in phenotypic gene may cause a change in the phenotype.
  - Typically, more than one gene is responsible for a phenotype.
- Genotype
  - The genetic makeup of an individual composed of one or more specific genes

• The genotype of an individual refers to its particular genetic make-up.

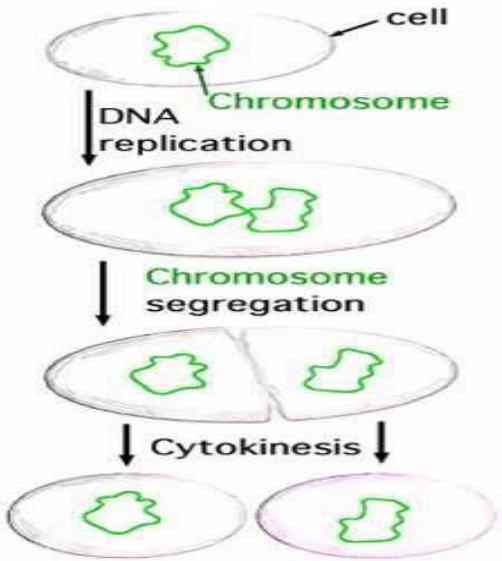
• The phenotype is the actual characteristics observed for an individual, or manifestation of genotype. (e.g. skin color).



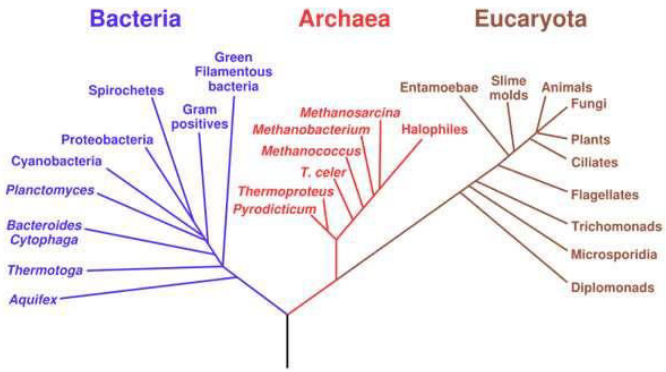
Increasing Genetic Diversity in Prokaryotes

Gene Transfer

- **Vertical** gene transfer – organisms replicate their genomes and provide copies to descendants. Passing on genes to descendants.
- **Horizontal** gene transfer – donor contributes part of genome to recipient that are not descendants; three types:
  - Transformation
  - Transduction
  - Bacterial **Conjugation**



Phylogenetic Tree of Life



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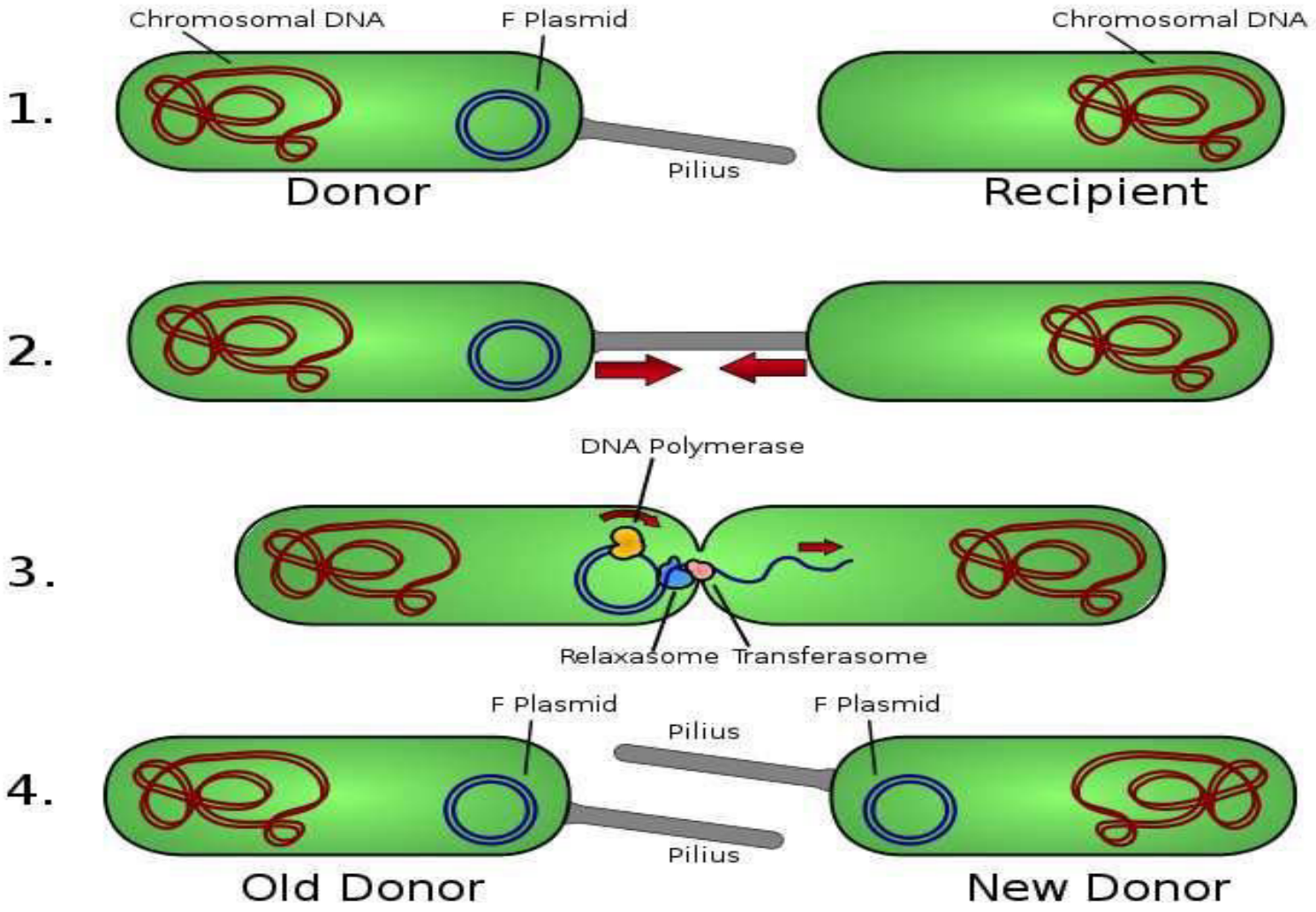
Mutation types

- Lethal mutation: results in death of the cell, and therefore cannot be propagated or studied
- Conditional mutation: One that is expressed only under certain environmental conditions; for example, a temperature-sensitive mutation
- Biochemical mutations: result in change in a biochemical pathway of the cell; for example, an auxotrophic mutation
- Spontaneous mutation: one that arises spontaneously due to error during DNA replication
- Induced mutation: one that has been caused by damage resulting from chemical or radiation treatment (mutagen)

Gene Transfer in Bacteria

- Conjugation
  - A process of gene transfer from a living donor cell to a living recipient cell
  - Typically, the donor cell will possess conjugative structures on its surface that attach the donor cell to the recipient cell. The conjugative structures will also mediate the transfer of DNA from the donor to the recipient.
  - The ability to conjugate is often encoded on a plasmid.
  - For example: In *Escherichia coli*, conjugation is mediated by the F pili that are encoded for by genes on the F plasmid.

Conjugation



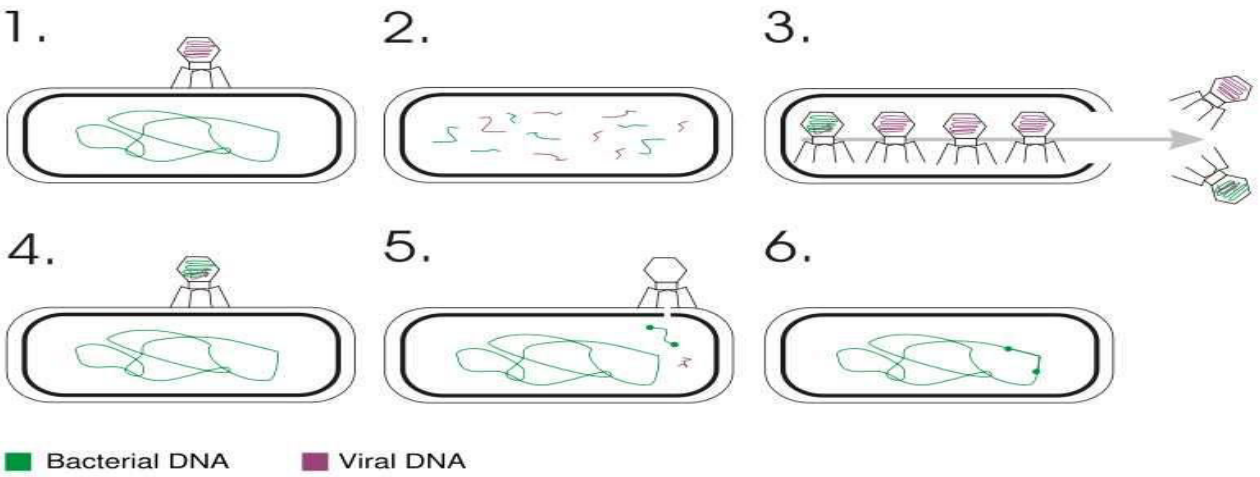
Gene Transfer in Bacteria

Transduction

- Transfer of genes from a donor cell to a recipient cell through a bacteriophage intermediate.
- Bacteriophage: A bacterial virus
- Bacteriophages have either lytic stage or lysogenic stages or both of them in their developmental cycle

• When a bacteriophage infects its host bacterium, it either integrate its DNA into the host chromsome and replicated every time the bacterial cell replicates (lysogenic) **OR** it replicates its own DNA and capsid protein within the infected host, reassembles thousands of new virus particles, and lyses the host cell to release the new viruses (lytic cycle).

Example: T4 phage of *E. coli*



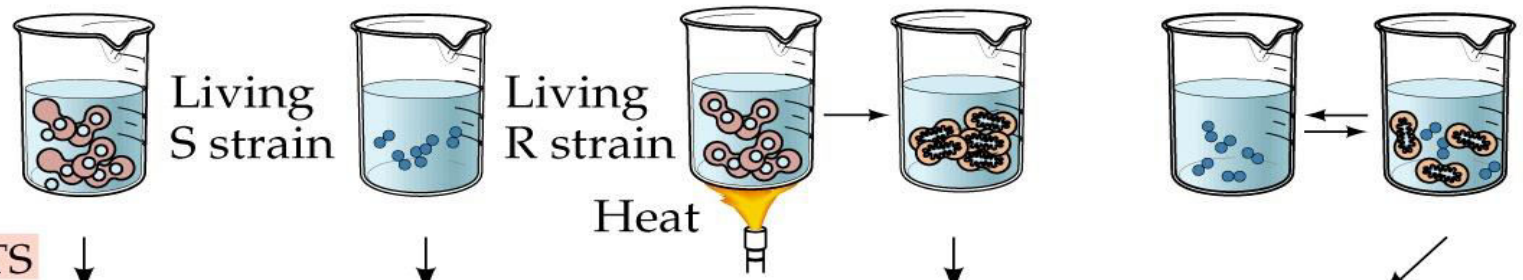
Gene Transfer in Bacteria

- Transformation
  - Transfer of isolated donor DNA (either chromosomal DNA fragments or plasmid DNA) to a recipient cell.
  - Successful transformation depends on the presence of double-stranded donor DNA molecules that are large enough, as well as cells that are competent for transformation

EXPERIMENT

Question: Can an extract from dead bacterial cells genetically transform living bacterial cells?

METHOD



RESULTS

Mouse dies	Mouse healthy	Mouse healthy	Mouse dies
Living S strain cells isolated from heart	No bacterial cells found in heart	No bacterial cells found in heart	Living S strain cells isolated from heart

Conclusion: A chemical component from one cell is capable of genetically transforming another cell.

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