Bio 11

Replication, transcription, and translation
History of the genetic molecule

• Is it protein?
• Griffith and his mice
• Avery’s experiments
• Hersey-Chase
• Chargaff’s rules
• Watson and Crick
• Meselson and Stahl
Watson and Crick with their DNA model
Rosalind Franklin

X-ray image of DNA
Meselson and Stahl Experiment

Original DNA double helix

DNA molecules after one round of replication

Conservative replication

Dispersive replication

Semiconservative replication
BASE SEQUENCE (DNA)
The 5' carbon on the ribose sugar defines the 5' strand end

The 3' carbon on the ribose sugar defines the 3' end

5' → 3' direction: The strand grows at its 3' end

Base (C, A, U, or G)

Ribose sugar

Phosphate
(b) Atomic model
(a) DNA synthesis begins at a single origin in the circular bacterial chromosome.

Strands of parent double helix

When the replication forks meet, replication will be complete.

(b) Parent DNA strands

DNA synthesis can begin at many origins in a eukaryotic chromosome.

Newly formed daughter strands are red

(c) Replication forks

Synthesis proceeds in both directions from an origin of replication.

Growth of forks
Eukaryotic Replication

Parental strands

Origin of replication

Parental strand

Daughter strand

Bubble

Two daughter DNA molecules
Primase binds to DNA and synthesizes an RNA primer.

When the primer is complete, DNA polymerase binds and synthesizes new DNA.

RNA primer

New DNA

Primase is released.
Synthesis of the leading strand is continuous.

The lagging strand is synthesized as Okazaki fragments.

The replication fork grows...

Most recently synthesized DNA

...and grows.

Okazaki fragments
DNA polymerase III elongates the leading strand.

Helicase unwinds the double helix.

Single-stranded DNA-binding proteins make the templates available to RNA primase and DNA polymerase III.

RNA primase makes primer.
RNA primase forms an RNA primer—DNA strands will form only from the 3’ end of a primer.

DNA polymerase III synthesizes the new Okazaki fragment, continuing until it encounters the primer on the previous Okazaki fragment.

DNA polymerase I steps in, hydrolyzes the RNA primer, and replaces it with DNA.

DNA ligase then catalyzes formation of the phosphodiester linkage that finally links the two Okazaki fragments.
(a) DNA proofreading during replication

During DNA replication, an incorrect base is added to the growing chain.
The proofreading proteins immediately excise the incorrect base.
DNA polymerase adds the correct base and replication proceeds.

(b) Mismatch repair

During DNA replication, a base was mispaired.
The mismatch repair proteins excise the mismatched base.
DNA polymerase adds the correct bases.

(c) Excision repair

A base in DNA is damaged so that it is not functional.
The excision repair proteins excise the damaged base and some adjacent bases.
DNA polymerase adds the correct bases.
BioFlix
DNA Replication

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Transcription in *Prokaryotes*
Central Dogma

(a) DNA → RNA → Protein

Information coded in the sequence of base pairs in DNA is passed to molecules of RNA. Information in RNA is passed to proteins.

(b) DNA ↔ RNA → Protein

The reproductive cycle of retroviruses adds a step: reverse transcription. Information in proteins is never passed to nucleic acids.
Central Dogma

DNA → Transcription (mRNA synthesis) → Translation (protein synthesis) → Protein
Differences between RNA and DNA

- DNA uses GATC
- RNA uses GAUC
- RNA is temporary copy of DNA
- Made by RNA polymerase
- RNA is single stranded, DNA is double stranded.
Basic steps of transcription

**Initiation**
- RNA polymerase binds to the promoter and starts to unwind the DNA strands.
- RNA polymerase reads the DNA template strand from 3' to 5' and produces the RNA transcript from 5' to 3'.

**Elongation**
- Rewinding of DNA
- Template strand
- Unwinding of DNA
- Direction of transcription
- Nucleoside triphosphates (A, U, C, G)
Basic steps of transcription
(a) A close-up view of transcription
(b) Transcription of a gene
An inducer is added to growth medium.
Operons

- Half of the genes in E. coli are grouped into operons.
- We will look at two- lac and trp
- Operons have a promoter, operator and structural genes.
Operon

Regulation of enzyme activity

The end product feeds back, inhibiting the activity of enzyme 1 and quickly stopping the pathway.

Regulation of enzyme concentration

The end product blocks the transcription of all five genes. No enzymes are produced, the enzyme concentration falls, and the pathway stops.
Catabolism of Lactose

In an *E. coli* growing in the absence of lactose, the repressor protein coded for by gene *i* prevents transcription by binding to the operator.
Lac operon

- Three genes controlled by this operon, transcription is polycistronic.
- Repressor is made at a second site regulated by another promoter.
- When lactose is not present transcription is repressed.
- Repressor will bind to operator blocking RNA polymerase.
Lactose present

Lactose induces transcription by binding to the repressor, which cannot then bind to the operator.

Inducer (lactose)

RNA polymerase binds

Transcription proceeds

As long as the operator remains free of repressor, RNA polymerase that recognizes the promoter can transcribe the operon.

mRNA transcript
Low Glucose

• Glucose is the preferred media, when glucose is present lactose is repressed.
• In the presence of plenty of glucose there is a lot of ATP. As ATP is reduced in the cell the level of cAMP increases. This increases the level of transcription.
• High glucose this inducer is absent.
When supplies of glucose are low, a receptor protein (CRP) and cAMP form a complex that binds to the promoter and activates it, allowing transcription of structural genes that encode enzymes for catabolizing the alternative energy source.
High glucose

RNA polymerase cannot bind.

Structural genes are not transcribed.

A cell that contains ample glucose and does not require energy from other sources contains little cAMP and little CRP–cAMP; in such a cell, the structural genes are not transcribed and the catabolic enzymes are not formed.
Trp operon

- Trp operon has five genes.
- trp is repressed by the repressor in conditions where trp is in high concentration.
- Trp does not bind well in low concentrations. Turns the operator on.
- Repressor also transcribed from another place.
Operator- synthesis of tryptophan

Tryptophan absent

DNA

Regulatory gene $r$ produces an inactive repressor.

mRNA

The repressor does not bind to the operator of the tryptophan operon.

Inactive repressor

RNA polymerase can thus transcribe the operon's structural genes into mRNAs that are translated into enzymes of the tryptophan pathway.

RNA polymerase

Transcription proceeds

$P_{lac}$ o e d c b a

DNA

mRNA transcript

Enzymes of the tryptophan pathway

e d c b a

Translation
Tryptophan present

DNA

mRNA

Inactive repressor

Active repressor

Corepressor (tryptophan)

Tryptophan binds the repressor, permitting it to bind the operator.

Active repressor binds to the operator. Transcription is blocked.

$P_{lac}$ o e d c b a DNA

RNA polymerase cannot bind.

Tryptophan blocks RNA polymerase from transcribing the structural genes and prevents synthesis of the enzymes of the tryptophan pathway.
Eukaryotic Transcription
The final transcription factor, TFII, binds DNA at the TATA box.

Another transcription factor joins it...

RNA polymerase II binds only after some transcription factors are already bound to DNA.

More factors are added...

...and the complex is ready to transcribe RNA.
DNA bending can bring an activator protein, bound to an enhancer element far from the promoter in linear DNA, to interact with the transcription-initiation complex.
DNA → Transcription → DNA–RNA → RNA processing → mRNA → Translation
RNA primary transcript

Coding region of primary transcript

This sequence is recognized by RNA cleavage enzyme.

A “cap” of modified GTP is added here.

This symbol indicates that a large piece of RNA is not shown. It may be thousands of bases long.

Processed primary RNA transcript

G cap

RNA is cut here and poly A “tail” is added.
Pre-mRNA

Cap

Exon  Intron  Exon  Intron  Exon

poly(A) polymerase

Recognition sequence

Cleavage of downstream sequences and polyadenylation

Adenyllic acid residues

poly(A) tail (100–250 residues long)
**pre-mRNA**

**Exons** (sequences that encode protein)

**Introns** (non-coding sequences)

**Mature mRNA**

Exons 1–4 are now adjacent, ready for translation.
A small nuclear ribonucleoprotein particle (snRNP) binds by base pairing between its RNA and the junction between the exon and intron (5’ splice junction).

A second particle binds near the other end of the intron (3’ junction). This base pairing targets the rest of the spliceosome complex for intron removal.
Primary mRNA transcript

A spliceosome forms because of interactions between snRNPs and other proteins.

A cut is made between the 5' exon and the intron.

The free 3' OH group at the end of the just-cut exon reacts with the 5' phosphate of the second exon, splitting off the intron and joining the two exons.

After the first cut at the 5' end, the intron forms a closed loop, like a lariat. This is done to avoid any reactions on the 5' end of the intron if it was allowed to remain free.

The 3' exon is cleaved and spliced to the 5' exon.

Mature mRNA

The excised intron is later degraded in the nucleus.

Translation

Protein
The process shown in the diagram is the central dogma of molecular biology, which describes the flow of genetic information from DNA to RNA to protein.

1. **DNA to RNA (Transcription)**: DNA is transcribed into RNA, forming a transcript that is then processed.
2. **RNA Processing**: The RNA transcript undergoes processing, which may include splicing, editing, and modifications to create the mature messenger RNA (mRNA).
3. **mRNA to Protein (Translation)**: The mature mRNA is translated into a protein by the ribosome.
Translation
Central Dogma Again

DNA strand

TRANSCRIPTION

RNA

TRANSLATION

Gene 1
Gene 2
Gene 3
DNA molecule

Codon

A A A C C G G C A A A A

U U U G G C C G U U U U
<table>
<thead>
<tr>
<th>First base of RNA codon</th>
<th>Second base of RNA codon</th>
<th>Third base of RNA codon</th>
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</thead>
<tbody>
<tr>
<td>U</td>
<td>UU</td>
<td>Phenylalanine (Phe)</td>
</tr>
<tr>
<td></td>
<td>UUC</td>
<td>Serine (Ser)</td>
</tr>
<tr>
<td></td>
<td>UUG</td>
<td>Leucine (Leu)</td>
</tr>
<tr>
<td></td>
<td>CU</td>
<td>Leucine (Leu)</td>
</tr>
<tr>
<td></td>
<td>CUC</td>
<td>Proline (Pro)</td>
</tr>
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<td></td>
<td>CUA</td>
<td>Glutamine (Gln)</td>
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<td></td>
<td>CUG</td>
<td>Histidine (His)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Glutamic acid (Glu)</td>
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<td>AAC</td>
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</tr>
<tr>
<td></td>
<td>UAG Stop</td>
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</tr>
<tr>
<td></td>
<td>AUG Met or start</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GU</td>
<td>Serine (Ser)</td>
</tr>
<tr>
<td></td>
<td>GUC</td>
<td>Arginine (Arg)</td>
</tr>
<tr>
<td></td>
<td>GUA</td>
<td>Arginine (Arg)</td>
</tr>
<tr>
<td></td>
<td>GUG</td>
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</tr>
</tbody>
</table>
(a) A simplified diagram of a ribosome

(b) The “players” of translation

- mRNA binding site
- tRNA binding sites
- P site
- A site
- Large subunit
- Small subunit

- Ribosome
- Growing polypeptide
- tRNA
- Codons
- Next amino acid to be added to polypeptide
Cap

Start of genetic message

End

Tail
**Initiation**

1. The small ribosomal subunit binds to its recognition sequence on mRNA, and the methionine-charged tRNA binds the AUG initiation codon, completing the initiation complex.

2. The large ribosomal subunit joins the initiation complex, with methionine-charged tRNA now occupying the P site.

Met is the abbreviation for the amino acid methionine.
Polypeptide

P site

mRNA

Anticodon

A site

Codons

1 Codon recognition

Amino acid

ELONGATION
1. Codon recognition
2. Peptide bond formation

ELONGATION
1. Codon recognition
2. Peptide bond formation
3. Translocation

- Polypeptide
- mRNA
- Anticodon A site
- Codons
- Amino acid

New peptide bond
mRNA movement

ELONGATION
1. Codon recognition

2. Peptide bond formation

3. Translocation

Polypeptide

Amino acid

mRNA

Anticodon

Codons

P site

Stop codon

New peptide bond

mRNA movement
**Termination**

**Release factor** binds to the complex when a stop codon is in the A site.

**Stop codon recognition:** A release factor binds to a stop codon exposed at the A site.

**Releasing the polypeptide product:** The release factor disconnects the polypeptide from the tRNA in the P site, freeing both the polypeptide and the tRNA.

The remaining components (mRNA, small ribosomal subunit, and large ribosomal subunit) separate.
Protein synthesis begins on ribosomes not attached to endoplasmic reticulum.

The signal sequence of amino acids is present

The signal recognition particle binds to a receptor protein in the membrane of the ER.

The signal recognition particle is released. The signal sequence passes through a channel in the membrane.